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G. P. CLINTON, Sc.D.

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CO-OPERATIVE POTATO SPRAYING IN 1917.

G. P. Clinton, Botanist.
L. F. Harvey, Farm Bureau Agent.

Nature of Experiments.

This is the second report on co-operative potato spraying, the first having been made by G. P. Clinton and F. E. Rogers, former County Agent of New Haven County, in the Station's Report for 1916, pp. 355–64. The experiments for 1917 were confined to home-made 4–4–50 Bordeaux, this having proven most practical in the previous experiments, and were merely to determine how efficient spraying was under ordinary farm conditions, and, as it turned out, in a year when blight was not especially troublesome. It was planned to give four to six treatments according to weather conditions and with the equipment at hand. The conditions varied so at the different farms where the experiments were conducted that the details are given under each. Thanks are due the owners for their aid in conducting the experiments, which were made at the following places: Arthur D. Clark's, Charles R. Treat's, and S. D. Woodruff & Sons', Orange; S. A. Smith & Son's, Clintonville; Whittemore's Estate, W. M. Shepardson, Manager, Middlebury.

Experiments were also planned at the farm of Robert Treat, Woodmont. Two sprayings were made by Mr. Treat, one about the 1st of July and the second on July 20th, but further treatments were omitted because of the dying condition of a large part of the field the last of that month. This unusual trouble may have been partly due to lice, but probably drought and unbalanced fertilization, factors that figured so prominently in the wilt and prematuring of fields in this State in 1918, were as much to blame. No yields were taken from this field, as evidently the two sprayings had not been of value under these conditions.

The first sprayings were made early in July at all the places before any signs of the blight had appeared. In fact the very first blight found that year in the State was on July 24th at South Manchester, and was not found in any of the sprayed fields until considerably later. Blight caused no very evident harm except in the Middlebury field, which was quite wet in spots. At the

Treat farm the vines were all dead from sun scorch before any signs of blight appeared. Their premature death during August was due to very hot weather on the last few days of July and the first of August. The damage caused by this hot weather made unnecessary the sprayings planned for the last of August, except at the Whittemore estate.

The results of spraying were determined by yields taken of 100 foot lengths, in comparable rows of the different plots, and the yields per acre figured from these by the method indicated in the previous report. No figures for cost of labor or supplies were kept, but those given in the 1916 report apply fairly well. The details of treatments, observations and results at each place follow.

At Arthur D. Clark's Farm.

Mr. Clark's field of three or four acres was very conveniently situated to the barn with water supply, etc. The field of Green Mountains was in fine shape when the spraying was started and was on high ground, somewhat rolling, with variable soil conditions, especially as regards retention of moisture. A one-horse Iron Age power sprayer, with single nozzles provided with strainer attachments for each of four rows, was used. The four sprayings were made as follows: 1st, July 6th; 2nd, July 16th; 3rd, July 27th; 4th, Aug. 13th. The sprayed plots were gone over twice, in opposite directions, at each spraying. In the first two treatments arsenate of lead paste, rate of 4 lbs. per 50 gals., was used in the Bordeaux for potato bugs, and the checks were sprayed at the same time and rate with the paste in water.

The plots were as follows:

Rows 1 to 4. Check.
Rows 5 to 36. Bordeaux (twice over).
Rows 37 to 40. Check.
Rows 41 on. Sprayed as desired by Mr. Clark.

The first twelve rows were planted with Maine grown seed, and the remainder with home-grown (one season) of same variety. On July 27th the vines of the Maine grown seed were distinctly larger and so more difficult to coat thoroughly with the spray than the home grown. Also on this date the effect of the poorer and more leachy soil in certain parts of the field began to show in the prematuring and yellowing of the vines, despite the fairly

good fertilizer applied. At the time of the fourth spraying, August 13th, some of the vines were dying prematurely, especially on the poorer part of the field, and more of those from home grown than from the Maine grown seed. There was no blight present. The hot weather of the last of July with resultant drought conditions in the leachier soil was entirely responsible for the trouble. There was little difference between sprayed and unsprayed plots. On Aug. 28th, one fourth of the vines were dead due to drought, with sprayed plots a little better than unsprayed. Found the first blight at this time on the low spot in the field but it appeared too late to cause much damage. Two rows of the Maine grown seed, from which Mr. Clark had cut six inches off the tops about two weeks before, were greener than the other vines in the rows on either side.

The potatoes were dug October 15th. The results of the yields are given in Table 1. From this it can be seen: 1st, That the Maine grown seed yielded better than the home grown, as was indicated by the appearance of the vines earlier; 2nd, That in each case the sprayed plots yielded better than the corresponding unsprayed plots; 3rd, That the average increase was about 18 bushels per acre, enough to slightly more than pay the cost of the spraying at the current price of potatoes per bushel (probably $15.00 clear profit); 4th, That the greatest difference between the sprayed and unsprayed plots was shown on the wet end of the field where the blight had been found on the foliage doing some damage.

At Charles R. Treat's Farm.

Green Mountain potatoes were planted here on two or three acres of sod land, and had the usual good fertilization and cultivation given by Mr. Treat. The sprayings were made with a one-horse 4-row Iron Age power sprayer with single nozzles provided with strainers. Part of the field was gone over once at each spraying with Bordeaux and part twice, the second time in the opposite direction. The sprayings were made on the following dates: 1st, July 2nd; 2nd, July 16th; 3rd, July 27th; 4th, Aug. 13th. In the first two treatments, arsenate of lead at the rate of 4 lbs. per 50 gals. was used in the Bordeaux, and in water for the checks.

The plots were as follows:

Rows 1 to 5. Check.

TABLE 1. DATA FOR THE A. D. CLARK FARM, ORANGE.

	Treatment.	Feet dug	Row	lbs. 1st.	lbs. 2nd.	lbs. Total.	No. rot.	Rate 1st.	bu. per 2nd.	acre Total
Dry end	Check. No Bordeaux..... (1)	100	2–3	70.0	17.5	87.5	0	169.4	42.4	211.8
Dry end	Twice over Bordeaux..... 4 times. (1)	100	7–8	72.0	18.5	90.5	0	174.2	44.8	219.0
Dry end	Twice over Bordeaux..... 4 times. (2)	100	34–35	58.0	13.0	71.0	0	140.4	31.4	171.8
Dry end	Check. No Bordeaux..... (2)	100	38–39	52.5	17.0	69.5	0	127.1	41.1	168.2
Wet end	Check. No Bordeaux..... (1)	100	2–3	84.0	13.0	97.0	25	203.3	31.4	234.7
Wet end	Twice over Bordeaux..... 4 times. (1)	100	8–9	101.5	12.5	114.0	18	245.6	30.3	275.9
	Totals, Check.....	300	2–3 38–39 2–3	206.5	47.5	254.0	25	166.6	38.3	204.9
	Totals, Bordeaux.....	300	7–8 34–35 8–9	231.5	44.0	275.5	18	186.7	35.5	222.2

(1) Maine grown seed. (2) Home grown seed.

Rows 6 to 20. Bordeaux, once over each treatment.

Rows 21 to 81. Bordeaux, twice over each treatment.

The field was low, quite level and fairly uniform in character. On July 27th the vines were in fine shape except a small spot in the twice over Bordeaux plot, where lice had caused some injury. On Aug. 13th, however, they showed one-half the foliage dead, so the treatment on this date did little good. By Aug. 28th they were four-fifths dead. No difference showed in favor of the sprayed plots, and no blight was found at any time in the field. There is no doubt that the vines were so severely injured by the three or four days of unusually hot weather the last of July and Aug. 1st, resulting in premature death during August, that the spraying had not yet begun to produce the beneficial effects that usually appear from the last of August on. The vines here suffered more from this hot spell than at any of the other farms. One row next the corn, which shaded it somewhat, kept green longer than the others.

TABLE 2. DATA FROM THE C. R. TREAT FARM, ORANGE.

Treatment.	Feet dug	Row	lbs. 1st.	lbs. 2nd.	lbs. Total.	No. rot.	Rate 1st.	bu. per 2nd.	acre Total
Check. No Bordeaux..... (1)	100	2–3	65.5	16.0	81.5	0	158.5	38.7	197.2
Once over Bordeaux 4 times..... (1)	100	7–8	56.5	15.5	72.0	0	136.7	37.5	174.2
Twice over Bordeaux 4 times..... (1)	100	21–22	53.5	13.5	67.0	0	129.4	32.7	162.1
Check. No Bordeaux..... (2)	100	3–4	73.0	13.5	86.5	0	176.6	32.7	209.3
Once over Bordeaux 4 times..... (2)	100	8–9	67.5	16.5	84.0	0	163.4	39.9	203.3
Twice over Bordeaux 4 times..... (2)	100	21–22	67.5	10.5	78.0	0	163.4	25.4	188.8
Totals, Check.....	200	2–3 3–4	138.5	29.5	168.0	0	167.6	35.7	203.3
Totals, Bordeaux once over.....	200	7–8 8–9	124.0	32.0	156.0	0	150.0	38.7	188.7
Totals, Bordeaux twice over.....	200	21–22 21–22	121.0	24.0	145.0	0	146.4	29.0	175.4

(1) West side, south end of rows. (2) West side, center of rows.

The potatoes were dug on Sept. 27th. The results of the yields are given in Table 2. From the results and the observations made, it is evident: 1st, That the spraying did no good; 2nd, This was due to the premature death of the vines resulting from the sudden hot spell in July and August, before any blight got started; 3rd, The trampling of the ground and vines in spraying resulted in a decreased yield, shown most markedly in the twice over Bordeaux plots. Ordinarily where the vines are not killed prematurely, this slight injury due to trampling is overcome later in the season and additional gain made through the greater vigor and longevity of the sprayed vines.

At S. D. Woodruff & Sons' Farm.

As at the other places the potatoes were Green Mountains, with the fertilization and cultivation as ordinarily given by the owner. The field was similar to that at the Clark farm, being somewhat

lower and perhaps less variable in soil conditions, though none the less rolling. The planting, made with a planter, showed quite a few misses giving an uneven stand in the rows in scattered places. The one-horse Iron Age power sprayer used here, while of the four-rowed type with a single nozzle to a row, had a somewhat stronger pump, that gave a more even distribution of spray material. The sprayings were made on the following dates: 1st, July 6th; 2nd, July 16th; 3rd, July 27th. The fourth spraying planned for Aug. 13th was omitted because the machine was not available. Poison for bugs was used in same way as at the other farms.

The plots were as follows:

Rows 1 to 16. Check.

Rows 17 to 56. Bordeaux, twice over each time in opposite directions.

Rows 57 to 60. Check.

Row 61 on. Sprayed as desired by owner.

On August 13th the field was somewhat weedy, and by Aug. 28th, the weeds were so large and abundant as to cause more or less damage. On neither of those dates did the vines show as much injury from the hot weather of July as did those at either the Clark or the Treat farm. This was probably due to better soil conditions for retaining moisture. Blight had not appeared in the field up to the last of August. Little difference was seen between the sprayed and unsprayed parts of the field.

The potatoes were dug on Sept. 25th. The results are shown in Table 3. The conclusions are as follows: 1st, That the sprayed plots gave a very slight increase over the unsprayed; 2nd, That there was more variability in yield between the two checks than there was between them and the Bordeaux plots, probably due to uneveness of stand and soil conditions, though we tried to avoid such in selecting the spots that were dug; 3rd, The few sprayings given, the weediness of the field, the absence of blight, etc., made conditions such that very favorable results from the spraying could not be expected.

At S. A. Smith & Son's Farm.

The Green Mountain potatoes at this place were sprayed the first time with a hand-power barrel pump placed on a wagon to which was attached a stationary nozzle arrangement for spray-

TABLE 3. DATA FROM THE S. D. WOODRUFF & SONS' FARM, ORANGE.

Treatment.	Feet dug	Row	lbs. 1st.	lbs. 2nd.	lbs. Total.	No. rot.	Rate 1st.	bu. per 2nd.	acre Total
Check. No Bordeaux..... (1)	100	14	81.5	15.5	97.0	8	197.2	37.5	234.7
Twice over Bordeaux 3 times..... (1)	100	19	72.0	15.5	87.5	0	174.2	37.5	211.7
Check. No Bordeaux..... (2)	100	58–59	64.0	9.5	73.5	6	154.9	23.0	177.9
Twice over Bordeaux 3 times..... (2)	100	51, 55	75.0	13.0	88.0	5	181.5	31.5	213.0
Totals, Check.....	200	14 58–59	145.5	25.0	170.5	14	176.1	30.3	206.4
Totals, Bordeaux.....	200	19 51, 55	147.0	28.5	175.5	5	177.9	34.5	212.4

(1) Rows next road. (2) Rows center of field.

ing four rows, each from a single nozzle. This worked so poorly that part of the rows were gone over three times to thoroughly coat them. The next two sprayings were made by hand with two lines of hose and were done more thoroughly than at any other place. The first two sprayings contained powdered lead arsenate at rate of 2 lbs. per 50 gals. Bordeaux, for the bugs, and the checks received similar amounts in water. The sprayings were made on following dates: 1st, July 6th; 2nd, July 17th; 3rd, Aug. 6th. The abundance of weeds made it undesirable to give the fourth spraying.

The plots were as follows:

Rows 1 to 4. Check.

Rows 5 to 48. Bordeaux, thorough hand treatments.

Rows 49 to 55. Check.

On August 6th the field was becoming very weedy, and on Aug. 30th the weeds were so large and numerous as to seriously interfere with the crop, except in six rows where they had been pulled on Aug. 6th. The vines suffered little from the July hot spell and on Aug. 30th were greener than at any of the Orange farms. The first check rows showed little blight on Aug. 30th; however, the second check rows, in a lower damper part of the field, showed more. The spray at this time had mostly washed off the vines.

TABLE 4. DATA FROM S. A. SMITH & SON'S FARM, CLINTONVILLE.

Treatment	Feet dug	Row	lbs. 1st.	lbs. 2nd.	lbs. Total	No. rot	Rate 1st.	bu. per 2nd.	acre Total
Check. No Bordeaux..... (1)	100	2–3	64.5	13.5	78.0	5	156.1	32.7	188.8
Twice over Bordeaux 3 times..... (1)	100	5–6	81.0	15.5	96.5	3	196.0	37.5	233.5
Twice over Bordeaux 3 times..... (2)	100	43–44	48.5	15.5	64.0	0	117.4	37.5	154.9
Check. No Bordeaux..... (2)	100	50–51	53.0	22.0	75.0	2	128.3	53.2	181.5
Totals, Check.....	200	2–3 50–51	117.5	35.5	153.0	7	142.2	42.9	185.1
Totals, Bordeaux.....	200	5–6 43–44	129.5	31.0	160.5	3	156.7	37.5	194.2

(1) Weeded. (2) Not weeded.

The potatoes were dug October 8th. The results are given in Table 4. From this experiment it is seen: 1st, That the sprayed vines gave a little better yield than the unsprayed, about enough to pay for the cost of the treatments; 2nd, That the part of the field kept thoroughly weeded showed greater difference in favor of the spraying and also in favor of weeding. We do not know why Bordeaux plot 2 gave a smaller yield than check plot 2, unless the former was from home grown and the latter from Maine grown seed which was used in this end of the field.

At Whittemore's Estate Farm, Middlebury.

This field of several acres of Green Mountains was on a hill side and part of one end was quite moist even in dry weather, so that in the early season the potatoes were nearly smothered out there. It had been in sod the previous year, top dressed in the spring, and when plowed under had been so very liberally fertilized that the soil was in unusually good condition. The vines were sprayed with the large two-horse, 4-row Iron Age power sprayer that had three nozzles to a row, one above and on either side. The power was sufficient to give a very misty spray that with slow driving coated the vines in fair shape with the one spraying that was given at each treatment. Arsenate of lead was used in the first two treatments and on the check for bugs. The sprayings were

made on the following dates: 1st, July 3rd; 2nd, July 11th; 3rd, July 19th; 4th, July 30th; 5th, Aug. 14th; 6th, Aug. 27th; 7th, Sept. 3rd.

The plots were as follows:

Rows 1 to 12. Bordeaux, once over.

Rows 13 to 16. Check.

Rows 17 on. Bordeaux, once over.

The hot weather did not hurt this field and it remained green much longer than any of the others. In fact at the time of digging, Oct. 16th, some of the vines were still green! One of the largest hills, the vine of which was unusually luxuriant, yielded seven large potatoes and two small ones, a total weight of five and a half pounds. The tubers on the whole were rather large, one of the largest weighing eighteen and a half ounces. The large yield and large size of tubers were due to the good fertilization and the extended growth period of vines resulting from the spraying. Blight eventually appeared in September and caused death of the foliage and rot of the tubers especially in the wet area, and to a less extent in the unsprayed drier part of the field.

The yields are shown in Table 5. An examination of this shows: 1st, That the spraying very considerably increased the yield in

TABLE 5. DATA FROM THE WHITTEMORE ESTATE FARM, W. M. SHEPARDSON, MANAGER.

Treatment.	Feet dug	Row	lbs. 1st.	lbs. 2nd.	lbs. Total.	No. rot.	Rate 1st.	bu. per 2nd.	acre Total
Once over Bordeaux 7 times....	100	10–11	124.5	11.5	136.0	3	301.3	27.8	329.1
Check. No Bordeaux.....	100	14–15	95.5	7.5	103.0	60	231.1	18.2	249.3
Once over Bordeaux 7 times....	100	18–19	143.5	8.0	151.5	11	347.2	19.4	366.6
Once over Bordeaux 7 times.... (1)	100	10–11	85.5	8.0	93.5	106	206.9	19.4	226.3
Check. No Bordeaux..... (1)	100	14–15	22.5	6.0	28.5	257	54.5	14.5	69.0
Once over Bordeaux 7 times.... (1)	100	18–19	34.0	6.0	40.0	253	82.3	14.5	96.8
Totals, Check.....	200	14–15 14–15	118.0	13.5	131.5	317	142.8	16.3	159.1
Totals, Bordeaux.....	400	(2) 10–11 (2) 18–19	387.5	33.5	421.0	373	234.4	20.3	254.7

(1) Unusually wet spot covering only small part of the field.

each case over the adjacent unsprayed rows; 2nd, This increase averaged 95 bushels per acre; 3rd, The increased yield was in part due to the prolonged life of the vines, and in part to preventing rot of the tubers; 4th, The spraying did not prevent rot in the very wet part of the field, where considerably over half of the tubers rotted though it helped somewhat even there.

General Conclusions.

These were potato spraying experiments, chiefly with horse-power sprayers, carried on under the ordinary operations at five different farms. The hot weather, of three days the last of July and the 1st of August, seriously interfered with the results by prematurely killing or injuring the vines in August in two of the fields, and to a small extent in two more. This rendered unnecessary the last one or two sprayings that had been planned. Blight caused no conspicuous injury except in one field. The spraying harmed rather than helped in one field, due to trampling of vines in spraying and the premature death of the vines from drought before the effect of spraying showed; two fields were benefitted about enough to pay for the cost of spraying; one field gave an increase about (18 bushels) slightly above the cost; one field, where blight was prevented, gave an increase (95 bushels) very greatly in excess of the cost of spraying.

FERTILIZER EXPERIMENTS WITH POTATOES.

E. H. Jenkins, Director.
G. P. Clinton, Botanist.

The following is an account of observations on the yields of potatoes as affected by different fertilizers, especially as regards potash, conducted by Mr. W. M. Shepardson, Middlebury, Conn., the writers making the weighings at digging time, and by Mr. F. C. Davis, of Somers.

The first lot, northwest of Mr. Bristol's house, was in turf the previous spring and had not been cultivated for many years. In 1917 it was plowed, dressed with 25 loads of manure to the acre, and the fertilizers named were put in the drill at the rate of 1000 pounds per acre. The rows were $2^{3}/_{4}$ feet apart. The Green Mountain potatoes were planted by machine on June 9th. The stand obtained was quite uniform.

The potatoes were dug on Oct. 19th, and the crop on the two central rows of each plot, 100 feet long, was weighed and sorted into firsts and seconds. These tubers were obviously scabby where ashes were used, and more scabby where 2000 pounds of ashes were used than where half that amount was applied.

The weights in lbs. of the potatoes on the 2 rows were as follows:

Fertilizers.	lbs. Firsts.	lbs. Seconds.	lbs. Total.	*Est. Total Bu. per acre
Essex 4–8–4 (1916 stock)	112½	40	152½	184.5
" 4–10–0	122½	37½	160	193.6
" 4–10–0, 1000 lbs. ashes per acre	132½	37½	170	205.7
" 4–10–0, 2000 " " " "	157½	22½	180	217.8
Buffalo 2–9–4 (Templeton)	117½	30	147½	178.5
Essex 4–8–4 (1916)	127½	35	162½	196.6
Bowker 3½–9–4 (1916)	122½	42½	165	199.7
Buffalo 2–9–4 (Templeton)	117½	35	152½	184.5
Buffalo 2–9–4 (Outside rows)	125	27½	152½	184.5

*Estimated on basis of an acre field 16 by 10 rods, rows running lengthwise of field, and 3 feet apart.

The most noticeable things are that the duplicates with Essex and Buffalo in different parts of field gave rather uniform results indicating uniformity in the soil of the field.

The addition of ashes to the Essex 4–10–0 increased the yield and 2000 lbs. increased it more than 1000 lbs., particularly in the No.

1 potatoes. The ashes however induced scab, though not enough to *seriously* damage market quality.

On a neighboring field planted to Green Mountain on June 8th in rows 2 ft. 10 in. apart, 2 rows each 150 feet long were dug in each plot. 1000 lbs. of the fertilizers were used and on some plots varying amounts of wood ashes were added.

	Firsts. lbs.	Seconds. lbs.	Totals. lbs.	*Est. of Total bu. per acre.
Essex 4–8–4.....	180	75	255	205.7
" 4–10–0, no ashes,.....	200	55	255	205.7
" " " " 1000 lbs. ash.....	165	70	235	189.6
" " " " 2000 " ".....	230	80	310	250.1
" " " " 3000 " ".....	250	60	310	250.1

*Estimated on basis of an acre field 16 by 10 rods, rows running lengthwise of field, and 3 feet apart.

Here 1000 lbs. ashes had no apparent effect, 2000 and 3000 lbs. ashes increased yield to same amount. Scab was more abundant where ashes were used than on plots where not used, but there was not much difference due to the different amounts used.

In neither field did the Essex 4–8–4, with potash, give a better yield than the 4–10–0 without it. However, in both fields the addition of a ton or more of wood ashes to the Essex 4–10–0 materially increased the yield, due either to the greater amount of potash or to the lime they contained.

Mr. F. C. Davis, of Somers, reports in 1918 more favorable returns from potash, where he used it alternating on seven strips of twelve rows each, in a 3–8–3 formula in comparison with a 4–10–0. The land was a gravelly loam in corn the previous year. The fertilizers were applied in three doses as follows: 800 lbs. before the first harrowing, working it in thoroughly with the stalks and plowing under, 400 in the planter and 600 at the time of the second cultivation.

There was no difference between the strips during the growing season until the last ten days, the potash strips remaining alive that much longer. The difference in yield was about 50 bushels per acre in favor of the potash fertilizer. This at the price the potatoes were sold was a gain of $75 per acre or, deducting the extra cost of this fertilizer, a net gain of $64.

INSPECTION OF PHAENOGAMIC HERBARIA FOR RUSTS ON RIBES SPS.

G. P. Clinton, Botanist.

Not infrequently one can find specimens of certain fungi easier by looking through a collection of flowering plants in an herbarium than by going out doors and looking for them on the living hosts. This is true of certain smuts and rusts and especially so if the desired hosts do not occur in one's vicinity but are found in a large phaenogamic herbarium near by. Thinking that such a search through a number of herbaria perhaps might throw light on the early occurrence of *Cronartium ribicola* in this country, the writer in 1916 and 1917 carefully looked over the specimens of *Ribes*, including *Grossularia*, in the following eastern herbaria: (1) Conn. Agr. Sta. Herb., (2) Conn. Bot. Club Herb., (3) Yale Univ. Herb., (4) N. Y. Bot Gard. Herb., (5) Columbia Univ. Herb., (6) Grey Herb., (7) N. E. Bot. Club. Herb., (8) U. S. Nat. Herb. Requests were also sent to several central and western herbaria that leaves containing suspicious rust specimens be sent the writer and several such were received from the following: (9) Mo. Bot. Gard. Herb., sent by Greenman, (10) Univ. of Wash. Herb., by Hotson, (11) Univ of Calif. Herb., by Jepson. No rusts, however, were found on specimens sent from the last two herbaria.

While this investigation did not throw any light on the occurence of *Cronartium ribicola* in this country, except what might be considered negative evidence, still information was obtained of its existence elsewhere and of the distribution, hosts, etc., of three other rusts in this country. We have thought it worth while to record here this data, together with some general remarks on the same. The figures in parentheses, following the name of the collector with each collection, refer to the herbarium, as numbered in the preceding paragraph, in which the *Ribes* were examined. Most of these fragmentary specimens containing the enumerated rusts are now in the herbarium of the Conn. Agr. Exp. Station.

Besides these rusts several other fungi were observed but no particular attention was paid to them. They included the following: *Gleosporium Ribis* (Lib.) Mont. & Desm., on *Ribes Lobbii*, Klickitat Co., Wash., 7 Au. 1897, Suksdorf (6); on *Ribes nigrum*, Mt. Lancaster, Coos Co., N. H., 25 Au. 1913, Pease (7). *Septoria*

aurea destruens E & E., on *Ribes aureum*, Mobridge, S. Dak., 28 Jl. 1907, Bailey (8).

Aecidium Grossulariae (P.) *Schum.*

This fungus has now been proven by infection experiments to be the I stage of *Puccinia Grossulariae* (Schum.) Lag. with its II and III stages on *Carex* sps. Whether or not all of the aecia on the various species of *Ribes* scattered over the country are the same thing can only be determined by further inoculation tests. Arthur (Journ. Myc. 8: 53. 1902.) has described another *Puccinia, P. albiperidia*, with its II and III stages on *Carex* and its I stage on *Ribes* sps., distinguished by its white or nearly white aecia (*Aecidium albiperidium*). The same author (Mycologia 4: 14. 1912.) thinks that *Uromyces uniporulus* Kern also has its aecia on *Ribes*. We have however grouped all the specimens reported here under *Aecidium Grossulariae* as they showed no evident difference. This rust occurs very commonly on herbarium material and in the immature stage resembles somewhat the II stage of *Cronartium ribicola*. However, one soon learns to distinguish it in this stage with the hand lens through the larger size of the aecia and the presence of pycnia. This rust was collected on eleven different hosts as follows:

On *Ribes americanum* (*R. floridum*):—New Haven, Ct., 27 My. 1879, Livingston (3); Oxford, Ct., 23 Je. 1901, Harger (2); Bellevue, Wisc., 27 My. 1882, Schnelle (6); Norway, Neb., 22 Je. 1893, Rydberg (6, 8).

On *Ribes bracteosum*:—Chilliwach Valley, B. C., 8 Jl. 1901, Macoun (8).

On *Ribes* (*Grossularia*) *Cynosbati*:—Jackson, N. H., 29 Jl. 1876, Allen (1); Orono, Me., 25 Au. 1913, Pease (7); Northumberland, N. H., 31 Jl. 1909, Pease (7); Manchester, Vt., 30 Je. 1898, Day (7); Colebrook, N. H., 13 Jl. 1907, Pease (7); Kingston, Ont., 5 Je. 1902, Fowler (8); New Harpersfield, N. Y., 8 Je. 1906, Toppin (8).

On *Ribes* (*Grossularia*) *divaricatum*:—Yellowstone, Neb., Au. 1854, Hayden (9); Chehalis Co., Wash., 10 My. 1897, (4).

On *Ribes* (*Grossularia*) *gracile*:—Near Minneapolis, Minn., My. 1891, Aiton (3).

On *Ribes* (*Grossularia*) *hirtellum*:—Berlin, Mass., 13 Je. 1915, Winslow (7); Clinton Co., Ia., 23 Ap. 1878, Butler (1); Foxcroft,

Me., 25 Je.,1894, Fernald (7); Dead River, Me., 19 Au. 1896, Fernald & Strong (7).

On *Ribes lacustre*:—Sherburne, Vt., 18 Je. 1899, (4); Pittsburg, N. H., 5 Jl. 1907, Pease (7).

On *Ribes prostratum*:—Mt. Washington, N. H., 24 Je. 1893, Greenman (9), 24 Je. 1898, Williams (7); Straits Belle Isle, Lab., 1 Au. 1910, Fernald & Wiegand (6); Orono, Me., 1 Jl. 1892, Fernald (7); Cumberland, Me., 29 My. 1902, Chamberlain (7); Mt. Mansfield, Vt. 2 Jl. 1897, Williams (7); Oakham, Mass., 12 My. 1912, Fernald (7); Adams, Mass., 4 Je. 1898, Churchill (7).

On *Ribes* (*Grossularia*) *rotundifolium*:—Ames, Ia., Hitchcock (9); Ia., 1871, (9); Clinton Co., Ia., 22 Ap. 1878, Butler (1).

On *Ribes* (*Grossularia*) *saximontanum*:—Platte Canyon, Natrona Co., Wy., 7 Je. 1901, Godding (9).

On *Ribes* (*Grossularia*) *setosum*:—Buffalo, Wy., Jl. 1900, Tweedy (3); Bozeman, Mont., 18 My. 1901, Jones (8).

Coleosporium ribicola (C. & E.) Arth.

This is a western rust of which the aecial stage apparently is not yet known. It is probably some Peridermium on the leaves of *Pinus* sps. The uredinial stage was first described in 1878 (Grev. **6**: 86.) by Cooke & Ellis from the Rocky Mts., on *Ribes* sp. Arthur (N. A. Flora **7**: 86. 1907) who placed it under the genus *Coleosporium* and described the telial stage, gives Rocky Mountains (*Colorado*) and *Ribes* (*leptanthum*) as the type locality and host. In 1885 Peck (Bull. Torr. Bot. Club **12**: 36.) again described this species on *Ribes* from New Mexico under the name *Uredo Jonesii*. It is interesting to note here that at least 24 years before Cooke & Ellis first described the fungus it was unknowingly collected by Bigelow (Botanist, Whipple Exp. R. R. Route Miss. R. to Pacific Ocean, Fort Smith to the Rio Grande in 1853–4) on *Ribes leptanthum* as shown by specimens taken from this host in the Gray Herbarium. Arthur in his monograph cites as hosts five species of *Ribes* from eight states. We list here at least eight hosts from ten different localities, as follows:

On *Ribes aureum*:—Nogal Canyon, White Mts., New Mex., 17 Au. 1901, Wooton (II, 4), (III, 8).

On *Ribes* (*Grossularia*) *hirtellum*:—Black Tail Deer Creek, Yellowstone Park, III, Au. 1884, Tweedy (3).

On *Ribes lacustre* (*R. echinatum*):—Cascade Mts., Wash., II, 10 Au. 1893, Allen (4).

On *Ribes* (*Grossularia*) *leptanthum*:—Fort Smith to the Rio Grande, Whipple Exp., III, 1853–4, Bigelow (6).

On *Ribes montigenum*:—Sandia Mts., Sandoval Co., New Mex., III, 3 Au. 1910, Wooton (8); Sandia Mts., Balsam Park, New Mex., II, 17 Ap. 1914, Miss Ellis (4).

On *Ribes* (*Grossularia*) *pinetorum*:—Chloride, New Mex., III, 12 O. 1909, Goldman (8).

On *Ribes triste* (*R. rubrum*):—Yakon Valley, 40 Mile River, II, 17 Je. 1902, Collier (8).

On *Ribes Wolfii*:—Sandia Mts., Balsam Park, New Mex., III, 28 My. 1914, Miss Ellis (4).

On *Ribes* (*Grossularia*) sp.:—Calif., III, 11 S. 1909, Rusby (4).

Cronartium ribicola F. deW.

The search through various herbaria for this fungus was to learn, if possible, whether it occurred in the New England states earlier than the first reported collections made in 1914, and whether it was native in Colorado on *Ribes longiflorum* as indicated by the collections of Bethel. Nothing was learned along either of these lines. The western *Cronartium* in its II stage was first collected on *Ribes longiflorum* by Bartholomew in Stockton, Kans., Aug. 22, 1892, but has never appeared there since. During the last ten years Bethel has collected it a number of times on the same host, in both stages, at Boulder, Denver, and also other places in Colorado where it appears to be native. Recent experiments by government investigators have shown these collections to be distinct from our eastern introduced species altho *Ribes longiflorum* is considered by some as a synonym of *Ribes odoratum*, a common host in New England for *Cronartium ribicola*. In our search of herbaria, however, we did not find any Cronartium on *Ribes longiflorum*, though we did secure *Cronartium ribicola* on three specimens, two from Europe and one from Asia, as follows:

On *Ribes aureum* (*R. leiobotrys*):—Cult. plants in Berlin, Germany, II, III, 5 My. 1893, Koehne (6).

On *Ribes* (*Grossularia*) *divaricatum* (*R. irrigum*):—Cult. plants in Berlin, Germany, 9 Au. 1895, Koehne (6).

On *Ribes* sp:—C. China, W. Hupeh, My. 1900, Wilson, no. 515, (4).

Puccinia Ribis D. C.

This fungus is found in Europe and America, but is apparently not very common here, at least the writer has never collected it in the field. It seems to have a northern distribution and has been reported from New York by Peck, Wisconsin by Davis, Minnesota by Hollway, and probably from other states, and was found on several hosts, but not on the variety reported here. Peck describes it as a new species, *Puccinia pulchella* in 1873. There is only the telial stage in this rust.

On *Ribes triste* var. *albinervium*: - Van Buren, Me., 24 Jl. 1893, Fernald (7).

INFECTION EXPERIMENTS OF PINUS STROBUS WITH CRONARTIUM RIBICOLA.*

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HISTORICAL CONSIDERATION.

Introduction of Blister Rust in Connecticut, etc. In an article dealing with heteroœceous rusts found in Connecticut, published by the Station Botanist (1 p. 394) in his Report for 1907, the statement was made that the blister rust was "liable to be introduced on white pine imported from Europe". In April, 1909, Mr. F. A. Metzger, a forester employed by Mr. C. F. Street at Wilton to set out a plantation of 10,000 three year old white pine seedlings imported through the State Forester from Germany, found fifty to

*In order that the reader may gain at the start a fair idea of the fungus discussed here, a brief outline of its life-history follows: White Pine Blister Rust is the common name for the first or I stage of a rust imported accidentally into America on seedling white pines from Europe. The rust enters the pines through the pores, or stomata, of the needles in the fall. Usually within a year or two after infection, during late summer and fall, lemon-yellow drops containing minute spore-like bodies called pycniospores, ooze out from yellowish (but eventually brownish) areas on the bark, forming the O or pycnial stage of the fungus. The use of these so-called spores has never been determined. The next spring (the last of April to the middle of June) after the appearance of the pycnia, the first true spore stage, called the I or aecial stage, appears. This forms small, white, fragile, irregular eruptions on the bark projecting for about a quarter of an inch. They soon break open at the top disclosing an orange-yellow dusty mass of spores, the aecial spores.

The aecial spores are blown by the wind, or possibly carried by insects to the leaves of the alternate hosts, which comprise the various currants and gooseberries belonging to the genus *Ribes*. Germinating under favorable conditions of moisture, these spores send their germ tubes through the stomata, chiefly situated on the under side of the leaves, and usually within 7 to 12 days give rise there to the II stage of the fungus, called the uredinial stage. This shows as small roundish pustules, smaller than the head of a pin, that contain the yellow semi-dusty urediniospores. These are washed over the leaf or carried to the leaves of same or other plants and, on germinating and gaining entrance to the tissues, produce similar pustules. Through the summer several generations of these urediniospores may be produced spreading the dis-

one hundred infected with an unknown disease. He brought these to the Station and the trouble was later identified by the botanist (2 p. 731), who was then absent in Japan, as the white pine blister rust. This was the first definite finding of the blister rust in this state and one of the first in America, as about the same time it was also found on imported seedlings set out in several other New England states and New York. It is quite probable that the disease was brought into Connecticut at an even earlier date, as several plantations from foreign stock had been started in 1907, while nurserymen and others, on whose premises the disease has since been found in two cases, had imported seedlings and trees much earlier. There is also considerable evidence to show that it was brought into some of the other states some years before it was definitely first found in 1909. In New York it was introduced at least before 1906.

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ease more or less widely by this repetition. The black currant is especially susceptible to attack.

From midsummer on, the III or telial stage develops on the *Ribes* leaves often appearing in the same pustules following the II stage. This last stage shows as minute reddish-brown hairs about 1 or 2 mm. long and more or less thickly covering the under surface of the leaves. These hairs are composed of teliospores adhering permanently together. They germinate immediately under favorable moisture conditions and give rise to germ threads bearing very small secondary spores, sporidia, that are blown by the wind to the pine needles, and there gain entrance as already indicated.

Infected seedlings before the I stage appears in the spring are usually detected by their bunched leaves and soft swollen stems. When the fruiting pustules appear they are quite conspicuous and the disease is easily recognized. After these disappear in late June, there remains only the roughened dead bark or a yellowish-green area of invaded smooth bark which will break out the next year, to indicate infection. Once the cambium is killed all around the stem the parts above die. Very young infected seedlings do well to live a year, but those two or three years old may live for several years. On older trees if the infection is at the ends of the twigs the damage is not serious, but once the main trunk becomes invaded the tree is doomed though it may remain in vigorous growth for several years and seem perfectly healthy on casual examination of the foliage.

Elimination of *Ribes* in the vicinity of seed beds, thorough spraying of the beds with Bordeaux after midsummer if grown in an infected region, use of uninfected seedlings only for planting, inspection of plantations from foreign stock for infected pines, destruction of all wild or cultivated *Ribes* in plantations and for 300 yards around the same, are precautionary measures advocated for this state.

Upon positive identification, the botanical and forestry departments, in co-operation with the U. S. Department of Agriculture, inspected during 1909 most of the larger plantations scattered over the state, with the result that the blister rust was found in small amounts in several of them. All of the infected trees found were destroyed. In 1910 most of these plantations were re-inspected and several smaller ones were inspected for the first time. About 645,000 out of the 740,000 imported trees that had been set out, were thus inspected once or twice during the two years. As no trees were found infected in 1910, the inspection was practically discontinued. An account of this work appears in the botanist's report for 1909-10.

In the spring of 1912, Mr. Walden, inspecting an importation of white pine from Holland at one of the local nurseries, found some of the seedlings badly infected, and upon identification as this rust by the botanist (**3** p. 347) Dr. Britton ordered them all destroyed. Since that year importation of white pines from countries where the disease occurs has been prohibited by a quarantine of the U. S. Department of Agriculture.

The first outbreak found in this State on *Ribes* species, the alternative host for the II and III stages of this fungus, was found by the senior writer (**3** p. 348) on a few black currants at the home of Mr. H. B. Birdsey at East Meriden, Conn., in October of 1912. The origin of this infection has never been determined, since infected pines have not been found in this general region, though the rust has usually appeared each year since on these bushes.

Nature of Recent Work. In 1916 the rust having become prominent in certain plantations in northeastern United States and Canada, and especially wide-spread on the *Ribes*, the U. S. Department of Agriculture, and the several interested states, undertook anew methods looking for its control within the infected areas, and quarantine laws have since been passed regulating the shipment of five-needle pines and black currants from these to other parts of the country in order to prevent its introduction into other states. This necessitated further work in Connecticut. The forestry department of this station in co-operation with the United States Department of Agriculture, undertook especially the practical work dealing with inspection and control. The botanical department, while also helping with this phase, took as its special

problem the scientific study of the rust in its various aspects. The work under the direction of the forester has been carried on by funds from the U. S. Department of Agriculture and state appropriations, while that by the botanical department from state appropriations alone. It is the purpose of the latter department eventually to publish an illustrated bulletin covering all its investigations.* The report here deals solely with certain interesting discoveries that have been made relating to the method of infection and early development of the blister rust in the white pine. It can now be definitely stated that infection takes place through the stomata on the needles rather than through the stems or buds as commonly supposed. A preliminary note of this work has already been published by the writers (**4** p. 15) in the Report of the Blister Rust Committee for 1918.

Previous Investigations. While Dietrick (**6** p. 287) as early as 1856 reported both the *Peridermium* on white pine and the *Cronartium* on *Ribes* species from Russia, he evidently did not recognize that they had any relationship to each other. Klebahn (**8** p. XLVIII) in 1888 was the first to prove this relation by inoculations, using the aecial spores from the white pine on the leaves of *Ribes nigrum*. Later he and other European investigators, and Spaulding (**19** p. 244) in America, abundantly verified this relation by the inoculation of a great number of *Ribes* species in similar manner.

When we turn to the inoculation of the white pine with the telial stage from the *Ribes*, however, thus completing the evidence of their relationship, we do not find that many investigators have reported experimental evidence. In fact, Klebahn (**10** p. 86) in 1905 seems to have been the first and only European investigator to report such successful inoculations (made in 1903). As early as 1890 he (**9** p. 62) had attempted inoculations but was doubtful as to the results, as the plants used may have already been infected. While there is no doubt that he did infect two white pines under bell jars in the fall of 1903 (**10**), by placing infected leaves of *Ribes nigrum* on a wire net over them, unfortunately

*This report will be published by the writers and Mr. E. M. Stoddard of this department, who has also co-operated largely with the forestry department in its control work. The writers are indebted to him for the photographs and photomicrographs with which this article is illustrated.

he left the pines out doors over winter and did not carefully examine them until the middle of the following June.

It was evidently then too late to determine the very first signs of infection and the manner in which it took place. He noted the yellow spots on the previous year's leaves, of which we shall speak in connection with our own experiments, and found the mycelium of the rust in these spots. He also found that two of the young stems were of a paler color, distorted, and bore at first juvenile leaves instead of the normal fascicles of five leaves. In July spermagonial drops began to appear on the infected branches.

Concerning the manner and place of infection he seemed in doubt, but makes the following remarks: "The fact that localized yellow spots were present in these needles does not necessarily mean that the mycelium arising through infection in the needle has in especially high degree the power to grow forth in the longitudinal direction of the needles, and to penetrate into the branch. Whether the bark of the young branch at the time of the maturity of the sporidia can be pierced by these is likewise doubtful. Therefore it may perhaps be presumed that the fungus can easily penetrate into the branch if the base of the needle is infected. Still another possibility is that the sporidia must infect the young buds for the next year." Klebahn further stated in 1918 (**11**) "For *Peridermium strobi* it is shown that the needles can become infected but not that the mycelium grows from the needles into the bark."

Spaulding (**16** p. 147) in January 1912 published the following brief note concerning his inoculations on white pine: "Greenhouse inoculations have been made upon young *Pinus strobus* with teleutospores secured by inoculation on *Ribes americanum* with aecidiospores borne upon imported trees of *Pinus strobus*. Inoculations thus made in November, 1910, are now beginning to give results. One each of the trees inoculated with wounds and without wounds, is now showing slight swelling such as is so characteristic of the blister rust disease." This statement indicates, as Spaulding has recently told the writers, that the inoculations were on the stems. He also said that none of the infected pines formed the aecial stage, but the pycnia, with exudation of pycniospores, were produced.

From the results of these investigations by Klebahn and Spaulding one is left in doubt whether infection takes place through the

leaves, the buds or the stems, and in what particular manner. Judging from other statements in literature on this subject, the general impression is given that it takes place through the stems. For instance, Fischer, of Switzerland, (**7** p. 435) states: "The accidiospores infect the *Ribes* leaves upon which first uredo then teleutospores appear; the latter germinate immediately and infect the young twigs of pines." Spaulding (**17** p. 6) says in one of his earlier papers in 1912, concerning infection: "The winter spores are blown from the currant and gooseberry leaves upon which they are produced to various parts of the white pines in the vicinity. There they stick to the bark of the young trees or branches and germinate. The branching threads of the fungus penetrate the inner bark tissues for some distance, but cause no external sign of disease until nearly a year after the time of infection". He makes about the same statements in his excellent bulletin (**15** p. 26) on the "Blister Rust of White Pine" published the year previously, and in 1916 he (**18** p. 14) states: "The teliospores falling upon bark of suitable age on a white pine may in turn germinate, penetrate the bark and grow in the inner layers during the incubation period already mentioned." Metcalf (**14** p. 3) in 1917 makes a similar statement as follows: "The pine blister attacks white pine seedlings or pines of any age that have needle-bearing twigs through which it can enter. The disease spreads from the twig" etc.

McCubbin, also, writes in 1916 (**12** p. 1) "It is believed that the fungus gains entrance by some wound in the twigs or branches, and from the point of entrance it grows rapidly up, down and around the branch in the soft outer bark". In an article written in 1917, however, he (**13** p. 95) questions the branch method of infection as follows:

"Only indefinite references to the method of infection of the pine by *Cronartium ribicola* have appeared in current literature. From these references one gathers the impression that infection takes place through the bark, and probably by way of wounds or abrasions. Having an opportunity for studying a considerable number of pine infections in 1916, some attention was given to this point and records were made of the origins of cankers where such origins could be determined.

"In most cases the determination was not difficult, owing to the fact that in a healthy pine branch the fungus spreads out from the court of entry in a very regular and equal manner, and that its progress is marked by swelling or discoloration or both, or else the cortical tissue is killed in

an equally radial fashion. By taking note of this habit one can readily locate the point of original infection in most cases, especially in the earlier stages.

"Very early in this study it became apparent that the chief mode of infection was by way of leaf fascicles through the so-called short shoots. In these pines, which were all healthy and which grew in situations where they were fairly free from accidents, wound infection played but a very small part.

"According to the tabulated results about 92 per cent. of these young blister cankers originate in leaf-bundle infection. This percentage includes only those cases where the point of origin could be confidently established, but it is highly probable that a large proportion of the number listed as undertermined should also find a place here, and it might not be overstepping the mark to ascribe at least 95 per cent. of these blister cankers to leaf fascicle infection.

"One may consider that the sporidia from the currant leaves are lodged among the bases of the needles and from this position can then attack the short shoot which bears these leaves."

PRESENT INVESTIGATIONS.

First Infections. On October 21, 1916, the senior writer placed infected leaves of *Ribes nigrum*, having the telial stage, over two crocks each containing three two-year old seedlings and two crocks each containing six one-year old seedlings of *Pinus strobus*. These were moistened, covered with bell-jars for several days and have been kept in the greenhouse ever since. Nothing suspicious showing during the first month or two, they were not carefully examined thereafter for some time. On June 14, 1917, the writers looked them over critically and in one of the crocks found two of the one year old seedlings showing conspicuous yellow spots which were not seen on the leaves of the other plants. These two seedlings also showed the bark slightly swollen and of a yellowish-green color. On July 14th one of the two year old seedlings, which had shown nothing very suspicious previously, was found to be oozing pycniospores in less than nine months after inoculation. Later a second of these two-year old seedlings died and microscopic sections showed pycnia. Several of the young seedlings were dead by this time, whether from rust or other causes was not determined. At the present writing two of two-year old and one of the one-year old seedlings are still alive, but show no signs of infection. Thus out of 18 plants at least 4 were definitely known to have become infected.

Sections of the needles through the yellow spots mentioned above showed abundance of mycelium which was also found in the swollen stems and because of this it was uncertain whether the infection had taken place originally through the leaves or stem. It was to settle definitely this point that inoculations in the fall of 1917 were again made. These plants were carefully watched from week to week to obtain the first indications of infection. The results obtained were verified and extended by other experiments made in the fall of 1918. Therefore the data given in the following pages are the combined results of infections made during these three years.

Methods of Inoculation.

In Greenhouse: Condition of seedlings. Practically all of the pine seedlings used in the experiments have been grown in crocks usually from three to ten in a crock, and have been kept ever since in the greenhouse of the Experiment Station. Some of these have been grown from seed and others were obtained from our own and a nearby nursery where blister rust has never occurred. No rust either on *Ribes* or white pines has ever been found in the vicinity of the Station. Therefore there is absolute certainty that any infections that have taken place are the results of our inoculations. Most of the seedlings used have been one, two or three years old, since for convenience and ease of infection these have proved most satisfactory. When we speak of one year old seedlings we mean those of less than a year's growth as they varied in age from four to eight months. Similarly by those two and three years old we mean in their second and third year's development. For the most part these plants have made excellent growth. As they have been kept in the greenhouse the year round they have not had the winter rest period of plants growing outdoors, but during late fall and early winter their growth has been noticeably retarded. Of course where infection has taken place in the fall these plants in late winter and early spring have shown the disease far in advance of those plants infected at the same time but kept outdoors through the winter. This forcing of the plants and the disease indoors must be taken into consideration, at least as regards time, when comparing with infections that take place in nature. It is evident from these experiments, however, that the rate of development of the disease is correlated with the vigor and rapidity of the growth of the plant.

Preventive measures. Unless it were desired to keep certain parts of the plant from becoming infected, the inoculation material was placed in and around the moistened plants which were kept covered with a bell jar in subdued light for several days. Where it was aimed to produce infections through definite parts, care was taken to inclose the inoculation material with damp cotton to prevent the sporidia from escaping, or else the inoculated part was left exposed and all the rest of the plant was protected by dry cotton or paper. Plate XXXVII, fig. 3 shows an example of the latter condition where spores had been placed on the young leafy shoots after the rest of the plant had been covered.

Parts inoculated, (a) Stems. The inoculations on the stems were made in two ways: 1st by cutting a slit in the stem and inserting the germinating telial column; 2nd by applying the telial material to the uninjured stem. In both cases the material on the part inoculated was wrapped with moist cotton. The cotton also served to indicate the place of inoculation later on. The stems used, especially on younger seedlings, were quite small (less than a quarter of an inch in diameter and were one or two years old) except in a few cases where the green stem of the present year's growth on larger plants was used.

(b) Buds. By unopened buds we mean the terminal ones and in an entirely dormant condition closely enwrapped with the enveloping brown scarious scales, with no green parts of leaves exposed. By open buds we included those expanded enough to show the developing leaves. In both cases especial care was taken to protect all other parts below from infection by the devices already mentioned.

(c) Leaves. With inoculation of leaves usually no particular effort was made to prevent general infection, since infection of the leaf was determined by the appearance of yellow spots. It was always possible through microscopical sections of these yellow spots to find the particular stoma or stomata through which entrance was gained. There were four different methods used: (1) placing infected leaves on the soil of the crock, or (2) over the plants themselves, or (3) still attached to stems stuck in the soil, and (4) sprinkling water containing telial material over the plants. In some cases attempts were made to inoculate special parts of the leaves as base, centre, or apex, such places being marked by a string tied at the point of inoculation to identify it for later

examination (Pl. XXXVII, fig. 2). Again, leaves of different ages were used, as those young and still growing and others one or two years old. Also the juvenile leaves, such as first appear on the seedlings (Pl XL, fig. 4) or under exceptional conditions on older plants, were inoculated in comparison with the ordinary leaves which develop in fascicles of fives on the plants after the first year.

In Petrie Dishes. Having been successful with the inoculation of *Ribes* leaves in moist Petrie dishes (4 p. 14) it was thought that this method might be applied to the pine needles, at least so far as showing place and method of entrance of the germinating sporidia. Both juvenile and fascicled leaves were used, the former on short shoots or on the entire seedling and the latter unattached or attached to the stem (Pl. XXXVII, fig. 1). Inoculations made in 1917 showed no apparent infection though the needles were kept in good condition for a month. Possibly the failure here was due to the fact that the leaves were all of the fascicled form and were kept in the diffused light of the laboratory. The next year the experiments were extended to young seedlings and the Petrie dishes this year were kept in the more direct light of the laboratory greenhouse which faces the east and is protected from southern exposure to the sun by a wing of the building.

Under Tent. Another method tried was the inoculation of pines under a tent, to approximate natural methods of infection without danger of spreading the spores. The tent was made of unbleached muslin stretched over a frame about four by eight feet and about six feet high at the ridge. A door on one side made the examination of the plants easy at any time. To this tent two black currant bushes had been transplanted to determine if the *Cronartium* was carried over on bushes infected the previous year. Nothing showing in this respect the currants were artificially inoculated with urediniospores the middle of the summer of 1918. On August 15, after the appearance of the telial stage, a wooden box containing numerous small two year old white pine seedlings was set in the soil under each bush. On October 2, about a dozen plants from each box were transferred in a bunch to crocks in the greenhouse to see if forcing the plants during the winter might have any effect upon the appearance of the disease. On September 28 a white pine about a foot high and at least five years old was also placed under each infected bush.

Out of Doors. In addition to the preceding, three pines about three or four feet high and six to eight years old growing in a sheltered spot on the Station grounds, and three trees, fifteen to twenty feet high and fifteen to twenty years old at the Whittemore estate in Middlebury, Conn., were also inoculated. In the case of the pines at the Whittemore estate there was a possibility of outside infection since *Peridermium strobi* had been found on certain of these trees. At both the Experiment Station and at Middlebury the method used for inoculation was to spray the branch with water and then to inclose it in a double paper bag in the inner one of which were placed loosely or sewed to the paper fresh *Ribes* leaves infected with the telial stage. A variation of this method was to spray the leaves with water containing the germinating teliospores before placing the bag over the branch. The paper bags were left on the branches from two to four weeks and in some cases even longer. Except for the longest periods, living in the bags did not seem to injure the leaves to any appreciable extent. These experiments at both places were carried on in 1917 and again on other branches or trees in 1918. Altogether in the two years nine branches at the Experiment Station and thirty-one at the Whittemore estate were inoculated.

General Results of Inoculation.

Out of Doors. Of the forty branches inoculated at the Experiment Station grounds and at the Whittemore estate twenty were made each year. Despite these many attempts not a single inoculation was successful so far as can be determined at the present time (July 9, 1919). Those made in 1917 were certainly old enough to show some sign of infection if successful. Close watch has been kept for indication of yellow spots on the leaves so characteristic of greenhouse infection, without any evidence of the same. Somewhat suspicious spots have been sectioned for decisive proof of the presence of the mycelium but also without result.

To some this might indicate that in nature infection does not take place through the leaves, but to the writers it is more of an indication that the methods of inoculation were faulty, since there is no evidence of any infection through the young stems or buds which were equally exposed with the needles. The age of the leaves could not have been the limiting factor, since leaves nearly two years old (about their age limit) were abundantly infected on

the young trees in the greenhouse. It is uncertain whether these apparent failures were due to rapid decline of the inoculating material after being placed in the bags, to unfavorable conditions of moisture or temperature, or to some other unknown factor. In our greenhouse experiments we have had great variations in results of inoculations due to conditions which we have not always been able to explain. We speak of these outdoor inoculations as failures since in the greenhouse experiments some sign of infection visible to the eye has always shown within one to six months after the inoculation if successful.

Under Tent. On October 2, at the time of the transference of part of the two year seedlings from the box under the tent to crocks in the greenhouse, no sign of infection was visible on the leaves. On November 8 a single very inconspicuous yellow spot, evident only under the lens, was found on one of the plants in the tent and this on sectioning showed the characteristic mycelial masses. A day or two previous similar spots had been found on four of the plants transferred to the greenhouse.

During the winter the seedlings in the tent showed little evidence of further development of the fungus. For example, on February 24 only a few spots were seen on the leaves. These were scarcely visible to the naked eye, being about one-fourth of a millimeter long, directly over the stomates and not encircling the leaf as yet. In comparison with the spots on the plants transferred to the greenhouse they were very much less developed. On May 24, however, these spots were more numerous and were evident to the naked eye, some of them encircling the leaf while others were scarcely visible. One plant showed fourteen leaves with these spots and the stem had already become invaded. A very large percentage of the plants apparently were infected. Unfortunately a number of the plants had been winter-killed and during June a severe hot period killed most of the remaining ones, only four being alive in July. This greatly interfered with determining just how far advanced these spots on out of door plants would be at this time of the year, but those that were present were not very striking. These experiments would indicate that infection in nature, which takes place in the fall, is very inconspicuous and may entirely escape detection at that time, and that the spots develop rather slowly during the spring, perhaps in cases not becoming conspicuous until the middle of July.

On the other hand the plants that were transferred October 2 to the greenhouse continued to develop, at first slowly during the late fall and early winter. By November 26 at least seven out of the fifteen plants in one crock and all eight plants in the other crock showed inconspicuous spots indicating infection. By January 28 the spots were more conspicuous, in cases six to ten showing on a single plant. At this time the spots were one-sixteenth of an inch long and barely encircling the leaf. In one plant a slight swelling and yellowing of the stem indicated infection there had already taken place. From February 20 to the middle of March *these spots became quite conspicuous*, usually one to four mm. long and entirely encircling the leaf. The average spot was about two mm. long. In cases where infections had taken place close together these golden-yellow spots were merging. By the last of March it was evident that nineteen out of the twenty-three plants transferred had these infections on the leaves and their appearance was in strong contrast to those that had been left in the tent during the winter, showing how the favorable greenhouse conditions had hastened the development of the fungus. By July 1 the spots were about 2–3 mm. in length, though by merging some were even 6 mm. long. However by this time the spots were past their prime as some of the leaves containing them were dying or drying up. At least one plant was dead and on some of the others the new shoots were killed. It seems evident that, while a few have formed pycnia, none will survive to form aecia.

The two large white pines placed in the tent on September 28 on the date of the last examination (July 8, 1919) failed to show any certain sign of infection. If such had taken place the yellow spots on the leaves have remained obscure. These plants of course were transplanted in the tent much later than the seedlings and were not directly under the bushes, as were the former, so that the conditions for infection were not so favorable.

In Petrie Dishes. Of a score or more of inoculations in Petrie dishes with the pine leaves, both of the fascicled and juvenile forms, we were successful in only two experiments. One of these was on very young seedlings and the other on shoots having juvenile formed leaves. In no case were we able to detect infection on the fascicled leaves. Even on the juvenile leaves the spots were so slight as to require careful inspection with a lens to

determine their presence. Four out of six seedlings in one case showed a few spots which were identified by microscopical sections as containing the rust. Of the five shoots in the other case, infection was positive in two and probably in two more. All these infections were first identified about five weeks after inoculation by which time most of the Petric dish cultures were usually in poor condition through molds, etc. These spots were first visible somewhat later than the earliest signs found in potted plants in the greenhouse. The length of time required after inoculation before infection shows makes this a rather unsatisfactory method for telial inoculation as compared with the same method for inoculation on *Ribes* leaves.

In Greenhouse. (a) Stems. In the two years, inoculations were made on thirty-five cut and fourteen uncut stems and not a single one of these forty-nine cases has shown any evidence that the inoculation was successful. This seems somewhat contradictory to the results obtained by investigators with other species and with the apparent result obtained by Spaulding with this method. We are not certain whether the latter used any precautions in preventing infection through the leaves. We do not wish to state positively that infection cannot take place through the stem, either wounded or unwounded, because we were unsuccessful in our attempts. Possibly the small size of the stems through which we attempted inoculations may have had something to do with the failure. There is no question that on very vigorous green shoots stomata occasionally occur and it is not impossible that the germ tubes of sporidia may gain entrance through them. Their small number and the difficulty of the sporidia sticking to the stem, however, would make the chance of infection slight as compared with that of the leaves.

(b) Buds. During the two years inoculations were made on the opened buds of twelve plants, seven of which were successful. On the unopened buds fourteen inoculations were made in 1917 and ten in 1918. Of these only two were successful, both made in 1917, and it is not certain whether these buds can be strictly considered as unopened. Our classification of buds as opened and unopened, too, in 1917 was not so clear cut as in 1918. In one of the plants the permanent slide shows, by the presence of a definite sclerotial mass some distance from the base, that infection took place through a scale which had become elongated,

green and needle-like. In the other plant evidence is lacking how infection took place as sections were not made until the tissues of the young stem had become thoroughly invaded.

From the results of these experiments it seems that infection can readily take place through opening buds or shoots which have exposed leaves containing stomata. On the other hand it seems quite certain that infection does not take place through an unopened bud closely enwrapped by the scarious brown scales. In some cases these scales, however, continue their development into modified leaves and, through the stomata of such, infection no doubt may take place as in the case cited above.

(c) Leaves. As has just been noted infection readily took place through the leaves. In fact so far as we can determine of the one hundred successful inoculations reported in Table 6 all, except in the two very doubtful cases already cited, were traceable to leaf infection. The very first sign of infection to the naked eye, visible usually about a month or two after inoculation, or even earlier with a hand lens, is a very small yellowish spot *centering in the lines of stomata* that run lengthwise of the leaf (Pl. XL, fig. 3). These infected leaves later showed *evident golden-yellow encircling spots* (Pl. XL, figs. 1–2) *which were filled with characteristic sclerotial masses of the fungus*, even when there was no mycelium in the stem. Eventually, however, it appeared there at the base of these infected leaves. Sections through the yellow spots also definitely proved that *infection took place through the stomata* as indicated by a characteristic substomatal vesicle directly below the guard cells. Details of infection and the nature of the substomatal vesicle will be discussed later. Suffice it to say here that there was always found in microscopic sections of the yellow spot, usually about the center and beneath a stoma, a substomatal vesicle with a primary hypha leading down to the sclerotial mass (Pl. XLI, fig. 2; Pl. XLIII, figs. 1–6). For example, in one leaf nineteen distinct yellow spots were counted and sections of this leaf showed at least twenty-one substomatal vesicles. In some cases the number of infections as indicated by yellow spots was obscured by the latter running together (Pl. XL, fig. 6) and in a few instances we found two substomatal vesicles below one stoma signifying two infections at this place.

So far as types of leaves are concerned infections were secured readily through both the juvenile and fascicled forms. The former

because of their flattened and somewhat grooved upper surface, thereby more readily retaining sporidia, are perhaps somewhat easier infected. As to age we succeeded in infecting leaves from the very young juvenile stage to those of fascicled form one and even nearly two years old. There did not seem to be any difference as to the part of the leaf involved (base, middle or apex) since infection readily took place all over (Pl. XL, fig. 1), except that the bases of the leaves may have afforded better chances for the sporidia to adhere.

Quite variable results were obtained with different methods of inoculation. The most successful was the one in which the *Ribes* stems with fresh infected leaves were stuck in the crock over the pines. This method approaches nearest to natural conditions. Of thirty-six plants in eight different crocks inoculated on Oct. 19 and 22, 1918, all but one were infected. Not only was this high percentage of infection secured but the many yellow spots on the leaves showed that the infections had been very numerous on each plant. Of these plants fifteen were three years old and twenty-one were seedlings of less than six months. Infections of the latter was so severe that at the present writing practically all have been killed. Fair results were also obtained where water containing telial columns was sprinkled over the plants especially those containing young shoots. The poorest results were given where the infected leaves were put on the soil under the pines, only one out of ninety plants becoming infected. Where such leaves were placed over the pines the results were better.

Percentage of infection does not alone explain the value of the method employed, since a single plant infected through a single leaf is not comparable to another plant infected through many places on numerous leaves. While the method of infection may have had considerable to do with the results obtained, the time of infection also seems to have been a factor. During the two years, on the whole, the best results were obtained during October. Infections, however, may take place at any time sporidia are produced in good condition, since we infected a plant in one instance in June.

Age of Plants. Out of two hundred and thirteen plants inoculated in 1917, one hundred and thirty-three were one year, and eighty were two years old; and of the twenty-four resulting infections eighteen were one year and six two-year old pines. In

TABLE VI—GREENHOUSE INOCULATIONS

Date	Total No. plants inoc.	Total inf.		Inf. Ribes leaves on soil.		Inf. Ribes leaves over plants.		Inf. Ribes leaves on soil and over plants		Inf. Ribes branches in soil and spreading over plants.	
		No.	per cent.	No. inoc.	No. inf.	No. inoc.	No. inf.	No. inoc.	No. inf.	No. inoc.	No. inf.
1916											
Oct. 21	18	4	22	..	..	..	..	18	4	..	..
1917											
Aug. 25	21	0	0	16	0	..	..	..	..	..	..
Aug. 27	9	0	0	..	..	..	..	..	..	..	..
Sept. 6	57	3	5	32	0	..	..	16	3	..	..
Sept. 12	11	0	0	..	..	..	..	..	..	..	..
Sept. 14	5	0	0	5	0	..	..	..	..	..	..
Sept. 24	9	0	0	9	0	..	..	..	..	..	..
Oct. 15	21	4	19	10	1	..	..	..	..	..	..
Oct. 16	8	4	50	..	..	..	..	..	..	..	..
Oct. 17	61	13	21	..	..	..	..	..	..	..	..
Nov. 1	11	0	0	11	0	..	..	..	..	..	..
Total...	213	24	11	83	1	0	0	16	3	0	0
1918											
June 13	1	1	100	..	..	..	..	..	..	..	..
Sept. 30	12	0	0	7	0	5	0	..	..	..	..
Oct. 1	48	9	19	..	..	..	..	..	..	..	..
Oct. 2	6	3	50	..	..	..	..	..	..	..	..
Oct. 5	11	1	9	..	..	..	..	11	1	..	..
Oct. 7	4	1	25	..	..	..	..	..	..	..	..
Oct. 8	10	2	20	..	..	..	..	..	..	..	..
Oct. 9	18	6	33	..	..	9	3	2	1	..	..
Oct. 10	5	2	40	..	..	..	..	2	0	..	..
Oct. 11	35	10	28	..	..	..	..	..	..	..	..
Oct. 19	28	28	100	..	..	..	..	..	..	28	28
Oct. 22	8	7	87	..	..	..	..	..	..	8	7
Oct. 26	2	2	100	..	..	..	..	..	..	..	..
Oct. 29	2	0	0	..	..	..	..	..	..	..	..
Oct. 30	3	0	0	..	..	..	..	3	0	..	..
Total...	193	72	37	7	0	14	3	18	2	36	35
Total for 3 yrs.	424	100	23	90	1	14	3	52	9	36	35

1918 of the one hundred and ninety-three pines inoculated (not including the twenty-three inoculated in tent and later transferred to greenhouse) fifty-eight were one year, thirty-six two years, and ninety-nine three years old. This year seventy-two plants became infected, twenty-nine of the one year, four of the two years and thirty-nine of those three years old. While these embraced the various methods of inoculation, they were not exactly the same

OF *Cronartium ribicola* ON *Pinus strobus*.

II spores on lef. spots on pine leaves		III spores sprayed over plants.		III spores sprayed over young shoots.		III spores on Buds.				III spores on Stems.			
						opened.		unopened.		cut.		uncut.	
No. inoc.	No. inf.	No. inoc.	No. inf.	No. inoc.	No. inf.	No. inoc.	No. inf.	No. inoc.	No. inf.	No. inoc.	No. inf.	No. inoc.	No. inf.
..	..	..	..	..	..	..	..	..	..	..	..	..	..
5	0	..	..	..	..	..	..	..	..	..	..	..	..
3	0	..	..	..	..	..	..	3	0	3	0	..	..
3	0	..	..	..	..	..	..	6	0	..	..	..	..
6	0	..	..	..	..	..	..	..	..	5	0	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	5	3	..	..	3	0	3	0
8	4	..	..	..	..	..	..	..	..	..	..	..	..
19	0	20	9	..	..	5	2	5	2(?)	12	0	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
44	4	20	9	0	0	10	5	14	2(?)	23	0	3	0
..	..	1	1	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
22	4	12	5	..	..	..	..	4	0	2	0	8	0
3	0	..	..	3	3	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	4	1	..	..	..	..	..	..	..	..	..	..
..	..	..	..	2	2	..	..	..	..	5	0	3	0
2	2	..	..	..	..	..	..	4	0	1	0	..	..
3	2	..	..	..	..	..	..	..	..	..	..	..	..
3	0	21	5	3	3	2	2	2	0	4	0	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	2	2	..	..	..	..	..	..	..	..	..	..
..	..	2	0	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..
33	8	42	14	8	8	2	2	10	0	12	0	11	0
77	12	62	23	8	8	12	7	24	2(?)	35	0	14	0

for the different ages of the pines used and so the results would be affected somewhat by the methods employed. However, each year the one year old pines gave the highest percentage of infection. For instance, in 1918, of the inoculated plants fifty per cent. of the one year, eleven per cent. of the two years and thirty-eight per cent. of the three years old became infected. These per cents. were much higher than those obtained in 1917, when only four-

teen per cent. of the one year and eight per cent. of the two years old were infected. In 1918 the conditions for securing infection were much improved by the use of infected *Ribes* leaves still attached to the branches, as one of the methods of inoculation, and by the presence on many of the three years old pines of new shoots, some of which had juvenile-formed leaves. For examples of abundant infections resulting, two hundred and eighty-four yellow spots were counted on thirty-eight leaves of a year old seedling; and one of the three years old pines showed fully as many similar spots on the fascicled leaves. The counts in the former case were made before it was apparent to the eye that the stem was invaded. The young growing seedlings and vigorous growing older plants, especially the younger parts, seem to take infection better than plants that are at a stand-still or somewhat sickly. This appears to be true of bright, dark green leaves as compared with those more or less speckled and not of so healthy a color.

Pycnia and Aecia. There is no question that the younger a seedling is when infected the less chance it has of survival. This is especially true of the very young seedlings, most of which failed to survive a year or even long enough to form the pycnial stage. The new shoots of the older plants, especially where infection took place through their juvenile form leaves, were often dead by the following summer. The number and place of infections, together with the vigor of growth, largely determine the life of the infected pine. Pycnia of the normal type and oozing pycniospores have appeared on a number of these infected pines even including some of those classified as one year old seedlings. The pycnia have been seen four months after inoculation but usually it was five or six months before pycnial drops appeared. Vigorously growing infected stems, when oozing pycnia, are especially subject to attack by chewing animals, apparently slugs, which conceal themselves in the damp soil of the crocks. This has no doubt interfered somewhat with the further development of the rust. In no case yet have aecia appeared on any of the infected plants. Of those infected in 1917 only five remained alive in February, 1919. They were the older and larger plants and it seemed quite possible that aecia might appear on them during the spring. However, by July, they had all been killed by the rust without its further development. Up to date none of the plants infected in 1918 has shown any signs of aecia, though producing

pycnia. However, as a number of these are of the largest size used and apparently growing vigorously, despite the evident infection, there is a possibility that they may produce aecia in 1920. See Table 6 for further details of greenhouse infection.

Other Species Inoculated.

During 1917–18 besides *Pinus strobus* four other five-needled species were inoculated, partly out-of-doors and partly in crocks in the greenhouse. Of these *Pinus excelsa* was inoculated nine different times; *Pinus flexilis*, three different times on five different branches; *Pinus cembra*, once on two different plants; and *Pinus koraiensis*, two different times on three separate branches. Inoculations with *Pinus excelsa* were all, except in two cases, in the greenhouse. The greenhouse plants were four and five years old, while the out of door plant, used both years, was about six to eight years old. The other three species were all out of door plants of the same age. Despite the fact that the rust has been reported on some of these hosts we did not in a single instance secure an infection.

Purely as a matter of curiosity, as successful results were not expected, inoculations were also attempted one or more times on the following two needled pines, all of which except *Pinus densiflora* were grown in crocks in the greenhouse: *Pinus austriaca*, *P. densiflora*, *P. resinosa* and *P. sylvestris*. In the case of *Pinus sylvestris*, five one-year old seedlings were used, as such young plants are most likely to become infected, if susceptible. As in the preceding species, none of these inoculations was successful.

DETAILS OF LEAF INFECTION.

Telia and their Germination. As already stated, the telial or III stage of the fungus may be formed on the *Ribes* leaves (Pl. XXXVIII, fig. 1) any-time of the year from June on, although in nature they usually begin to appear the latter part of July and are most conspicuous in September and October. They develop more or less thickly on the under side of the leaves as short hairy growths about one or two mm. in length (Pl. XXXVIII, figs. 2–3).

As seen individually under the microscope the reddish-brown telial hairs (Pl. XXXVIII, fig. 4) are found to be composed of a

solid column of spores. These spores are considerably elongated lengthwise of the column, although at the ends they are shorter and broader. The more cylindrical ones (Pl. XXXIX, fig. 1) are 37-60μ x 14-16μ while the shorter apical ones are usually 27–36μ x 18–21μ. Longitudinal sections show that they are formed in chains, those of the interior adhering more loosely together, but often breaking joints so that the telial column is rather permanent although with pressure it may separate into chains or single cells (Pl. XLIV, figs 1–2). Colley (5 p. 637) gives a comprehensive description of the telia in his excellent paper on the microscopic structure of the blister rust, so that further details are omitted.

The telial spores germinate, *in situ*, anytime after maturity when conditions are favorable (Pl. XXXVIII, figs. 5–6). Apparently each spore may give rise to a promycelium which is sent out from its side as a growth filled with protoplasm, ordinarily reaching a length about that of the spore (Pl. XXXIX, figs. 1–2). This promycelium soon is divided by septa into four uninucleated fertile cells filled with protoplasm and a semi-empty basal cell. (Pl. XLIV, figs. 5–6). From each of the fertile cells (Pl. XXXIX, fig. 3; Pl. XLIV, figs. 7–10) arises a prominent pointed sterigma about the length of the cell. When fully developed, the sterigma forms at its tip (Pl. XXXIX, fig. 4) a swelling into which as it grows passes the entire protoplasm and the nucleus of the cell, forming a spherical sporidium about 10–12μ in diameter (Pl. XXXIX, fig. 5). The sporidium when mature, is easily separated from the narrowed tip. Variation from the normal type of germination of the telia is shown where instead of a promycelium a mycelial-like thread is formed (Pl. XLIV, figs 11–12). Another abnormal type is shown when the cells of the promycelium round up and become separated as kind of sporidia-like bodies (Pl. XLIV, fig. 13). These variations are probably produced under unfavorable conditions of aeration such as are often obtained in van Tieghem cell cultures.

The sporidia are comparatively short-lived and germinate immediately under favorable conditions of moisture. Usually a single germ tube is sent out (Pl. XXXIX, fig. 6; Pl. XLIV, fig. 16) tapering somewhat towards the tip. In cultures this germ tube may reach a length several times that of the spore and may be more or less curled (Pl. XLIV, figs. 18–19) and rarely gives rise to a short branch (Pl. XLIV, fig. 22). Occasionally sporidia are found

which have two or three germ tubes developing (Pl. XLIV, figs. 15, 17, 21, 22).

Often a sporidium instead of forming an infection tube gives rise to a tapering sterigma about twice its length, on the tip of which is produced a secondary slightly smaller sporidium (Pl. XXXIX, fig. 7; Pl. XLIV, figs. 23–25). As apparently only a single one is produced, this formation of a secondary sporidium is probably to tide it over unfavorable conditions of infection. These data were obtained partly from the germinating telia *in situ* and partly from those placed in watch crystals and van Tieghem cells.

Infection through Stomata. When a sporidium is blown to a leaf no doubt it germinates in the manner as described above by sending out a germ tube. Contrary to the general belief this does not penetrate the epidermis but gains entrance through a stoma. So far it has not been possible to verify this statement by a direct microscopical examination showing the sporidium germinating on the outside of the leaf and the germ tube pushing between the guard cells of the stoma into the air chamber beneath. The reasons for this are that not sufficiently young infections, those less than a week old, have been examined, and the exceedingly great difficulty in locating points of infection, since there is no external evidence to guide one within three or four weeks after this takes place, by which time the delicate sporidium and its germ tube outside the stoma are obliterated.

The indirect evidence, however, is sufficient to prove that the germ-tube of the sporidium passes down between the guard cells of a stoma. The youngest stages in which we have been able to find infection was fifteen days after the sporidia had been placed upon the leaves. In this, as well as in numerous older stages where yellow spots indicated the general point of infection, there was always evident just beneath the guard cells (Pl. XLI, fig. 1) a characteristic substomatal vescicle, mention of which has already been made. We have never seen in any of our sections evidence that the germ tube penetrated directly through the cuticle into or between the epidermal cells. In fact we do not recall having seen any hyphae in the epidermal cells. On the other hand whenever we have sectioned a leaf through a small yellow spot, we have been able to obtain at least under one stoma the substomatal vescicle. This substomatal vescicle we consider to be an inflation of the germ tube immediately it has passed the guard

cells into the air chamber. There is always a beak-like elongation passing from the upper end of the vesicle and often projecting quite up to the central bulge of the guard cells (Pl. XLIII, fig. 6) where evidently the germ tube entering into the leaf becomes most constricted and is most likely to be severed.

Substomatal Vesicles. A substomatal vesicle is a characteristic swelling of the mycelial thread never found except just beneath a stoma. As already stated it always ends above in a short beak projecting up between the guard cells. This beak (Pl. XLIII, figs. 1–6) varies from 2 to 22 μ in length according as the section passes directly through the centre of the substomatal vesicle or to one side. It ends in a sharp point where the guard cells approach nearest together and below joins directly to the centre of the substomatal vesicle by a base about the usual width of a hypha. The substomatal vesicle in general is ovate to elliptical and is elongated in the direction toward the stomal pore. The general size of the vesicle is about 7–11 μ by 10–13 μ. This substomatal vesicle at least at first is filled with protoplasm, and has a single nucleus (Pl. XLIII, figs. 1–3). The beak and wall of the vesicle often become somewhat thickened.

Primary Hyphae. From the lower end of the substomatal vesicle there develops a thread (Pl. XLIII, figs. 1–5) about 2.5 to 3.5 μ wide which usually grows straight down toward the base of the air chamber. Sometimes it turns to one side and comes in contact with the parenchyma cells there, thus forming a shorter tube. This we call the primary hypha because it is the first representation of the mycelium within the leaf and is distinguished from the other hyphae in the immediate vicinity by its comparatively straight and rarely septate tube. Accordingly as it first comes in contact with parenchyma cells at the side or base of the air chamber, we have found it to vary from 9 to 40 μ in length. Once it comes in contact with a parenchyma cell a branch proceeds from its side and by the narrow penetrating tube enters within the cell to form the characteristic finger shaped haustorium (Pl. XLIII, fig. 1). A source of food supply now being assured secondary hyphae of the mycelium as described later are formed.

With the penetration of the aecial and uredinial spores through the stomata in *Ribes* we have found a swelling outside the stoma as well as beneath it. Whether or not such a swelling occurs in the germ tube of the sporidium on the pine we cannot state.

We have interpreted these external swellings (appressoria) not only as hold fasts but also as reservoirs of food for favoring successful and rapid entrance into the leaf. Likewise the substomatal vescicle not only acts as a hold fast which would prevent the germ tube from being pulled out through the stomatal pore because of greater width but also as a storehouse of food for the rapid development of the primary hypha.

Mycelium. The primary hypha having formed a haustorium begins the development of the mycelium. This (Pl. XLIII, figs. 4-5) differs from the primary hypha by the curled character of the threads which are also frequently branched and septate. The cells of the mycelium are usually much longer than wide, though in this respect there is considerable variation. Usually they vary from 2 to 6μ wide by 16 to 21μ long. The greatly branched and curled hyphae (Pl. XLIII, fig. 6) soon begin to form compact masses in which it is impossible to trace the individual threads. In the meantime some of the hyphae have penetrated the host cells by haustoria.

Haustoria. The haustoria enter the host cells by a very narrow penetrating tube which bores through the cell wall and after gaining entrance into the cell assumes its normal diameter of about 3 or 4μ. In general the haustoria (Pl. XLIII, fig. 4) are enlongated, finger-like bodies filled with protoplasm and may reach a length of 50μ or more. In the leaf two nuclei have been noted in one haustorium, though in the stem the haustoria are uninucleate. The usual tendency of the haustorium is to extend toward the center of the cell where the nucleus is situated. There seems to be a sort of attraction by the nucleus since the haustorium frequently forms an abrupt bend (Pl. XLIII, fig. 6) and partially encircles the nucleus or makes a depression in it, but apparently does not penetrate its membrane. The haustoria usually are simple but occasionally have one or two branches (Pl. XLIII, fig. 7 j) which may be more or less curled and mycelium-like with an occasional septum.

Sclerotia. The curled hyphae eventually form a very compact cellular mass much like sclerotia (Pl. XLIII, fig. 7m). The chief differences between these and typically free sclerotia consist in their greater variation in size and shape, and the lack of any differentiated bounding layer. The appearance of these sclerotial masses varies largely according as the section of the leaf is transverse or longitudinal. In the former (Pl. XLII, fig. 1) they are

much like normal isolated sclerotial bodies though frequently there seems to be some coalescing of separate masses. In general they are nearly spherical but with occasional irregularities due to coalescence. The size of the sclerotia depends upon their age. Three months or more after infection, with the yellow spots on the leaves very conspicuous, they may extend from epidermis to epidermis and destroy the substomatal vescicle, the evidence of the point of infection. Some of the larger sclerotia thus reach 400μ in diameter. They begin their development in the intercellular spaces causing pressure against the host cells, and such cells being enclosed in the sclerotial mass are finally disintegrated though occasional traces of them may be detected. Distortions of the vascular system and other tissues of the leaf may be seen as the result of this exaggerated growth of the mycelium. The color of the yellow spots as seen by the naked eye is due to the destruction of the chloroplasts in the cells of the host rather than to any pigment in the walls or protoplasm of the fungus. The parenchyma cells immediately surrounding the sclerotial masses contain chloroplasts with a sickly yellowish appearance. In other words the endochrome evident in the mycelium directly producing the spore stages is not found here.

In longitudinal sections (Pl. XLI, fig. 3) the sclerotia more frequently have the appearance of a compound structure or of individual sclerotia running together. This is due to the laminate structure of the leaf, as seen in longitudinal sections, where the fungus forms individual masses in the various intercellular chambers and eventually coalesce through continued growth.

The function of these sclerotia is somewhat in doubt. It was first thought that they might be the beginning of the pycnial stage, but no evidence of the development of pycnia on the surface of the leaf above them has ever been seen. The fact that they begin their development in the fall and that in the fully matured and old sclerotia in midsummer there is some evidence of slight disintegration of the internal cells, leads us to believe that they act as storehouses to insure the penetration of the fungus through the vascular system of the leaf into the stem. The stem penetration at times may prove a slow process especially when the point of infection is near the tip of the leaf. In such a case the fungus in the sclerotial stage may remain more or less quiescent during the winter and the final penetration of the stem be delayed until the

next spring or early summer. Such a sclerotial mass would insure final penetration much more certainly than a slight mycelial growth.

Invasion of Vascular Region. Once a sclerotium is developed in the tissues of the leaf it may encroach on the vascular system; that is, while the sclerotium at first is entirely in the mesophyll, it may eventually break through the endodermis (Pl. XLII, fig. 2) and be partially enclosed by the same. As sections are taken below the yellow spots the sclerotium gradually fades out of the region of the mesophyll and becomes limited to larger or smaller sclerotial masses in the parenchyma of the vascular region. Very soon all evidence of sclerotium and mycelium disappears from the mesophyll. Haustoria may invade the endodermal or parenchymatous cells of the vascular region. If the sclerotial masses reach the xylum side of the bundle small sclerotial masses or mycelial strands gradually find their way to the phloem.

Sections through the perfectly green leaf some distance below the yellow spot of infection now show the fungus entirely limited to this position within the vascular system. In cross sections of the leaf made still further down it is found that the mycelial strands become very limited in number, seldom branched and grow almost entirely in the longitudinal direction toward the base of the leaf on the phloem side of the bundle. If a single point of infection happens to occur near the tip of the leaf and sections are thus made far below it, it is almost impossible to distinguish the fungus (Pl. XLII, fig. 3) from the phloem since the hyphae in cross sections are so similar in size (3–5μ), shape and staining as scarcely to be differentiated from the smaller phloem cells. In the larger phloem cells haustoria have been seen and in some of the smaller ones their contents sometimes look like mycelial strands.

Invasion of the Stem. As the mycelial strands, running lengthwise in the needles in contact with the phloem, reach the base of the leaf, they begin to go out again between the cells of the mesophyll which in this region, however, is not so definitely marked off by the endodermis. Growing beyond the endodermal cells the fungus finally reaches down into the cortex developing haustoria abundantly in the host cells as it proceeds. Once within the stem the mycelium grows uniformly throughout the bark and causes a stimulus in its growth. This is manifested in seedlings of one to three years old by slight swelling of the stem and upon the green

younger parts by a general yellowish discoloration, these being the first evidence to the naked eye of stem invasion. Only rarely, however, have we found a distinct discolored spot on the stem at the base of the leaf fascicle through which the infection took place.

The mycelium now becomes very abundant in the young tissues of the stem. If the infected leaf happens to be situated on a stem a year or two old invasion is not so rapid or prominent to the naked eye as when it is situated on green parts of a very young growing stem. Entrance here seems to be almost certain death to the terminal parts before the end of the summer. Heavy infection of the stem before the leaves have made their full growth causes a stunting and bunching of the same (Pl. XL, fig. 5), a characteristic which is employed in plantation or seed-bed inspections for identifying the fungus before the appearance of its fruiting stage. In very young stems the swelling and discoloration may be accompanied by a bending of the terminal part (Pl. XL, fig. 4).

Heavy infection through many leaves checks in part the later development of the fungus, since the invaded shoots may be killed before the fruiting stages are formed. However, if this is only a lateral shoot, or if the plant is of some size the fungus may yet form its fruiting stage elsewhere. In none of our sections have we found that the mycelium directly invades the growing point, it usually being some distance below where the cells have about reached their normal size.

We have occasionally seen cases where the fungus evidently went up from the invaded stem into the new leaves situated on it and at the base of these, or even isolated a short distance above, formed yellow spots quite similar to those of the primary infections made through the stomata. Such spots seen late in the season might possibly be mistaken for original infection areas. We are not sure, either, that primary infections may not occasionally form secondary isolated yellow spots on the leaves below the point of entrance. We are certain, however, most of the yellow spots that have appeared on our infected plants are primary infection areas, even when many showed in a leaf, since they have appeared simultaneously a comparatively short time after the infection took place.

Appearance of Pycnia and Aecia. While we cannot state, from our experiments, the time of the development of the pycnia in outdoor infections, we presume that this usually takes place sometime during the early summer succeeding the fall infection, since this was the case with Klebahn's experiments (9). In our greenhouse infections however they have shown as early as February, and on young stems of sufficient diameter have formed slightly elevated yellowish and eventually reddish-brown areas similar to those occurring in nature. On these evident pycnial drops with normal pycniospores have appeared. Preceding development of pycnia microscopical sections show an exaggerated development of mycelium in the cortex slightly below the epidermis and from this mass are developed the erect, closely packed, fertile, pycnial threads, from the ends of which are abstricted the pycniospores as has been described in detail by Colley (5 p. 629).

In nature the aecial stage develops from more deeply imbedded sclerotial masses in the year following the appearance of pycnia. Unfortunately in none of our indoor infections has this stage been produced, so that details of the time and manner of its appearance cannot be given here.

Cycle of Development. From the sum total of our observations and experiments we may summarize the development of the fungus as follows:

1st year. Infection occurs from late summer to late fall through the leaves, producing at most very inconspicuous yellowish spots at the point of invasion. In rare cases it may be that these spots develop more conspicuously and invasion of the stem takes place before winter sets in.

2nd year. During spring and early summer the yellow spots on the leaves become more or less conspicuous; later there is invasion of the stem causing slight swelling and discoloration and possibly in certain cases pycnia are produced.

3rd. year. There is further swelling of the stem and possibly stunting of the leaves with pycnial development during the summer or (in case pycnia were found the previous year) with aecial formation in the spring.

4th. year. There may be formation of aecia. In cases of slight or localized infection, especially in hardened tissues, it may be that the formation of pycnia and aecia is delayed for even a longer time.

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EXPLANATION OF PLATES.

Plate XXXVII.

Methods of inoculating leaves of white pine with blister rust, *Cronartium ribicola*.

Fig. 1. Inoculated fascicled leaves in a Petrie dish.

Fig. 2. Leaves inoculated at definite points, marked by strings, under battery jar.

Fig. 3. Young terminal shoots inoculated under bell jar, parts below protected by covering of cotton and paper.

Plate XXXVIII.

Telial stage of the blister rust on currants.

Fig. 1. *Ribes nigrum* with telial stage on under surface of leaf. Slightly reduced.

Fig. 2. Same as fig. 1, showing portion of leaf surface slightly magnified and telial columns quite evident.

Fig. 3. Portion of fig. 2, still more highly magnified, about 7 diameters.

Fig. 4. Two telial columns, dimly showing cellular structure, extending from fragment of leaf at base. Magn. about 70 diam.

Figs. 5-6. Portion of telial columns sending out germ threads; fig. 6 also showing a fully developed promycelium with sporidia. Magnified about 135 diams.

Plate XXXIX.

Photomicrographs of the development of the promycelium from telial spores. All magnified about 760 diams.

Fig. 1. A single telial spore sending out a germ thread, the beginning of the promycelium.

Fig. 2. A nearly fully grown promycelium growing out of a cell of the telial column.

Fig. 3. Similar to fig. 2, but with promycelium septate and a single sterigma developed.

Fig. 4. Similar to fig. 3 but sterigmata forming young sporidia on their tips.

Fig. 5 An isolated promycelium with two empty fertile cells bearing fully developed sporidia, the other two filled with protoplasm and just starting the formation of the sterigmata, and the empty sterile basal cell.

Fig. 6. Sporidia just starting to germinate.

Fig. 7. A sporidium germinating and forming a secondary sporidium.

Plate XL.

Infected leaves and seedlings of white pine from greenhouse inoculations showing characteristic golden-yellow spots, etc.

Fig. 1. Fascicled leaves taken from a pine (No. 623) about 10 months after inoculation, showing a few of the several hundred infection spots that developed within four or five months after the inoculation. Slightly reduced.

Fig. 2. Juvenile leaves from an inoculated pine showing single infection spots. Slightly magnified.

Fig. 3. Leaf showing origin of very young infection spots directly over stomatal rows. Magnified about 18 diams.

Fig. 4. Infected seedling pine with juvenile leaves showing swelling, discoloration and bending of invaded terminal shoot.

Fig. 5. Artificially infected pines showing bunching and dwarfing of young fascicled leaves.

Fig. 6. Pine leaf showing how partially developed, closely-placed infection spots may run more or less together. Magnified about 12 diams.

Plate XLI.

Photomicrographs of cross and longitudinal sections of infected pine leaves. a-e, host cells; f-i, fungous cells: a, epidermis, b, stoma, c, guard cells, d, cells of mesophyll, e, vascular system, f, substomatal vesicle, g, primary hypha, h, mycelium, i, sclerotium.

Fig. 1. Cross section showing a substomatal vesicle and primary hypha beneath a stoma, and further in fragments of the mycelium out of focus. Magnified about 400 diams.

Fig. 2. Portion of fig. 1, more highly magnified, about 800 diams.

Fig. 3. Longitudinal section showing sclerotial masses on either side of vascular system. Magnified about 60 diams.

Plate XLII.

Photomicrographs of cross sections of infected pine leaves, showing leaf structure and sclerotial stage of rust. Magnified about 120 diams. a-e, host-cells; f-g, fungous cells: a, epidermis, b, mesophyll; c, endodermis, d, phloem side of bundle, e, xylem side of bundle, f, sclerotial mass of rust, g, vascular invasion threads.

Fig. 1. Sclerotial mass of rust in mesophyll to one side of vascular system.

Fig. 2. Sclerotial mass within endodermal sheath of vascular system.

Fig. 3. Mycelium of rust limited to a few invasion threads on phloem side of bundle shown by the darker stained cells.

Plate XLIII.

Cross sections of infected leaves of *Pinus strobus*. Magnified about 450 diams. a-f, host cells; g-m, fungous cells: a, epidermis, b, stoma, c, guard cells, d, cells of mesophyll, e, chloroplasts, f, nucleus, g, substomatal vesicle, h, substomatal beak, i, primary hypha, j, haustorium, k, mycelium, l, nucleus, m, sclerotium.

Fig. 1. Section of leaf showing a stoma with a substomatal vesicle below it and a haustorium developed from the primary hypha. Inf. no. 617 (2); inoculated Oct. 11, 1918, killed Nov. 8, 1918. Slide 11/8/18/(2). 2.

Fig. 2. Section of leaf showing a stoma with a substomatal vesicle having an elongated beak and the straight primary hypha. Pine inoculated Oct. 8, 1918, killed Nov. 9, 1918. Slide 11/9/18. 2.

Fig. 3. Section of leaf showing a stoma with substomatal vesicle cut somewhat diagonally and the long primary hypha. Inf. no. 617 (2); inoculated Oct. 11, 1918, killed Nov. 19, 1918. Slide 12/19/18/(1). 3.

Fig. 4. Section of a leaf showing a stoma with a substomatal vesicle below it and the primary hypha in contact with a host cell and developing mycelium. Inf. no. 617 (2); inoculated Oct. 11, 1918, killed Nov. 19, 1918. Slide 12/19/18/(1). 1.

Fig. 5. Section of leaf showing part of a stoma with a substomatal vesicle beneath and the long primary hypha ending in curled hyphae encircling a host cell. Inf. no. 617 (3); inoculated Oct. 11, 1918, killed Dec. 19,1918. Slide 12/19/18 (2). 3.

Fig. 6. Section of a leaf showing a stoma and a substomatal vesicle with a very much shortened primary hypha and a well developed mycelium. Inf. no. 626; inoculated Oct. 19, 1918, killed Jan. 6, 1919. Slide 1/6/19 (3). 3.

Fig. 7. Section through a small sclerotial mass in a pine leaf. Inf. no. 624 (2); inoculated Oct. 19, 1918, killed Dec. 17, 1918. Slide 12 17/18 (4). 16.

Plate XLIV.

Drawings of fresh material of telial stage. Figs. 1-2, 6, 11-13, magnified about 450 diams.; figs. 3–5, 7–10, 14-25, magnified about 650 diams. a. nucleus of teliospore, b. germ tube of teliospore, c. sporidium, d. fertile cells and e. sterile basal cell of promycelium, f. germ tube of sporidium, g. secondary sporidium, h. sterigma.

Fig. 1. Part of chain of teliospores taken from middle of column.

Fig. 2. Part of chain of teliospores taken from apical end of column.

Fig. 3. A teliospore with a single germ tube starting to form the promycelium.

Fig. 4. A teliospore with two germ tubes starting.

Fig. 5. A fully grown promycelium with four fertile cells and one sterile basal cell.

Fig. 6. A promycelium with cells starting to form sterigmata.

Fig. 7–10. Promycelia with mature sterigmata and sporidia in various stages of development; some of the fertile cells not yet germinating.

Figs. 11–13. Unusual types of germination of telial spores—showing in fig. 11 a branched germ tube, in fig. 12 a simple wavy germ tube, and in fig. 13 the fertile promycelial cells rounding directly into sporidia-like bodies.

Figs. 14–25. Sporidia in different stages and types of germination; 14, 15, 17, 21, 22, sporidia with more than one germ tube; 16, 18, 19, 20, sporidia with single germ tubes; 23–25, sporidia forming sterigmata with secondary sporidia.

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Fig. 1.

Fig. 2.

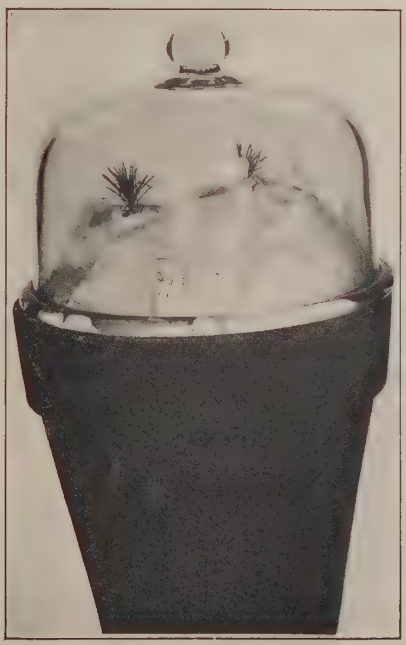


Fig. 3.

METHODS OF INOCULATION.

Fig. 1.

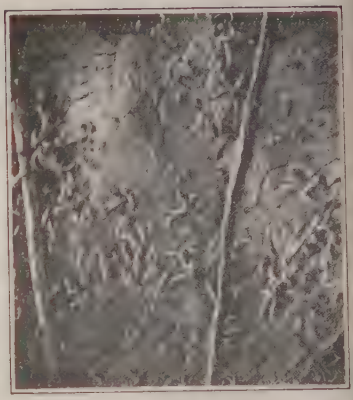


Fig. 2.

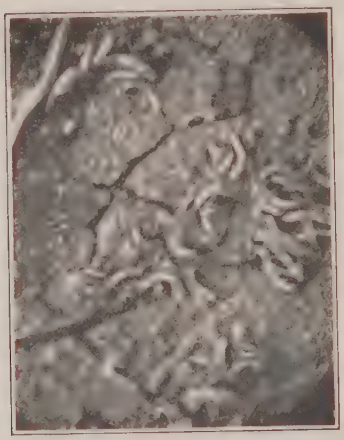


Fig. 3.

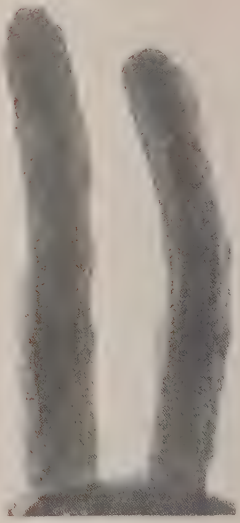


Fig. 4.

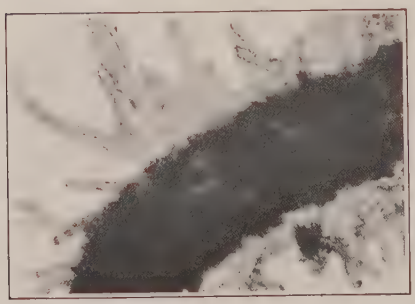


Fig. 5.

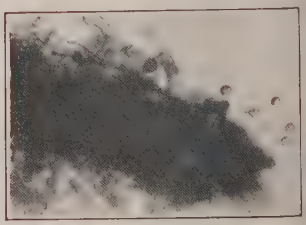


Fig. 6.

TELIAL OR III STAGE ON RIBES.

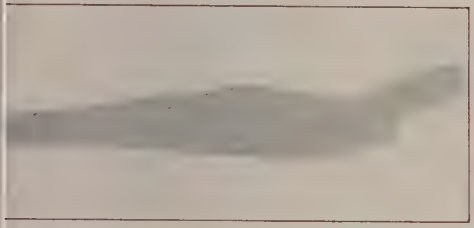


Fig. 1.

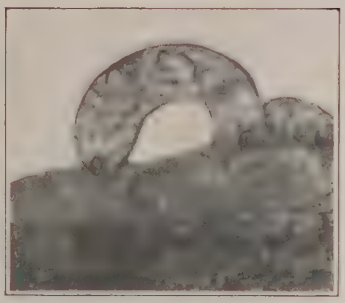


Fig. 2.

Fig. 3.

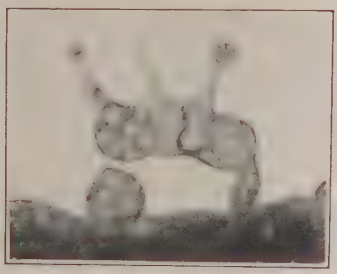


Fig. 4.

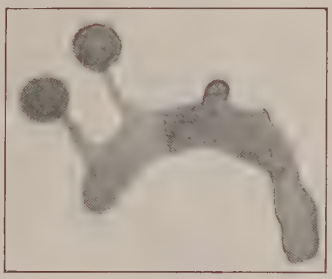


Fig. 5.

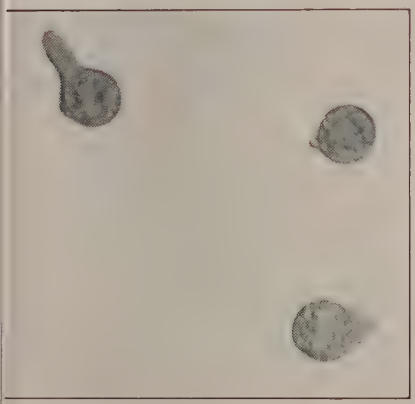


Fig. 6.

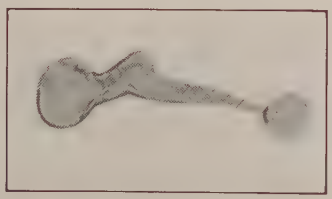


Fig. 7.

GERMINATION OF TELIAL STAGE.

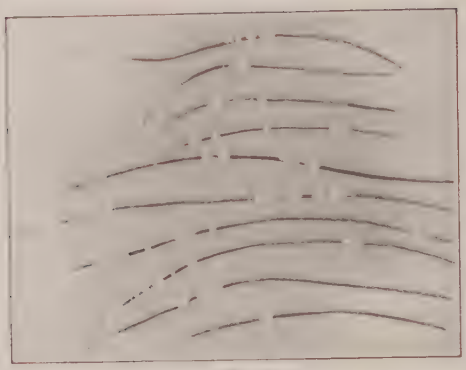


Fig. 1.

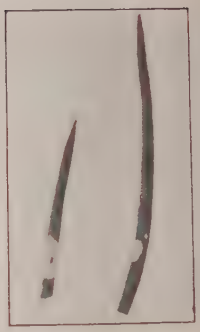


Fig. 2.

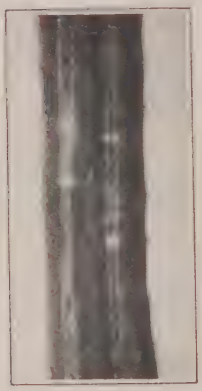


Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

INFECTED PINE LEAVES AND STEMS.

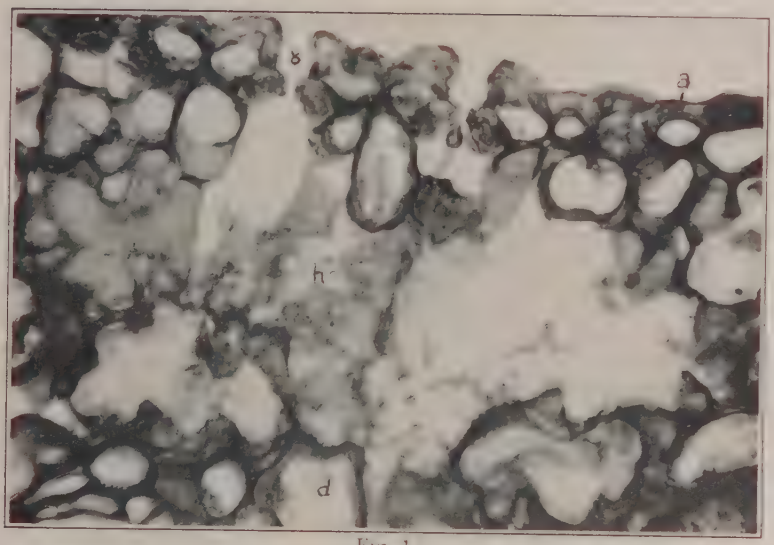


Fig. 1.

Fig. 2.

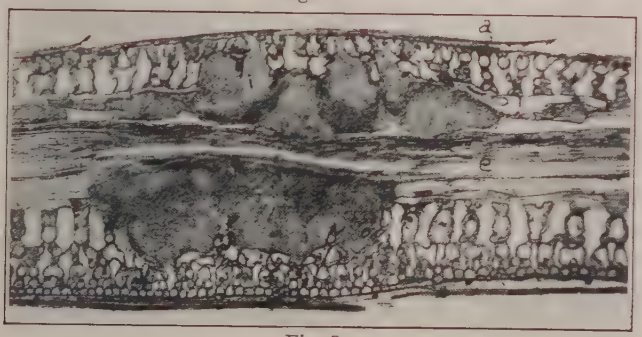


Fig. 3.

CROSS AND LONGITUDINAL SECTIONS OF INFECTED PINE LEAVES.

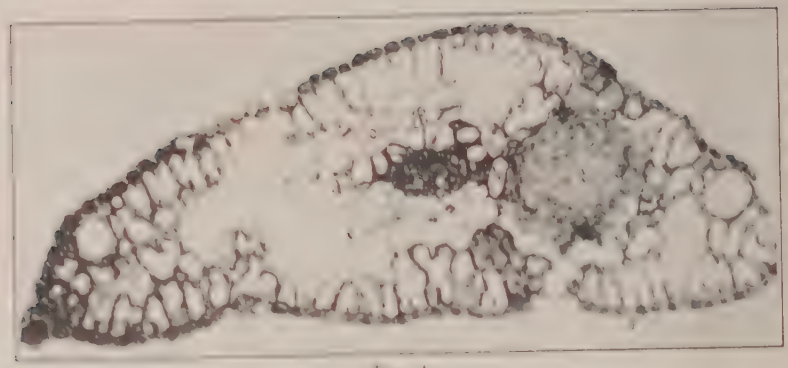


Fig. 1.

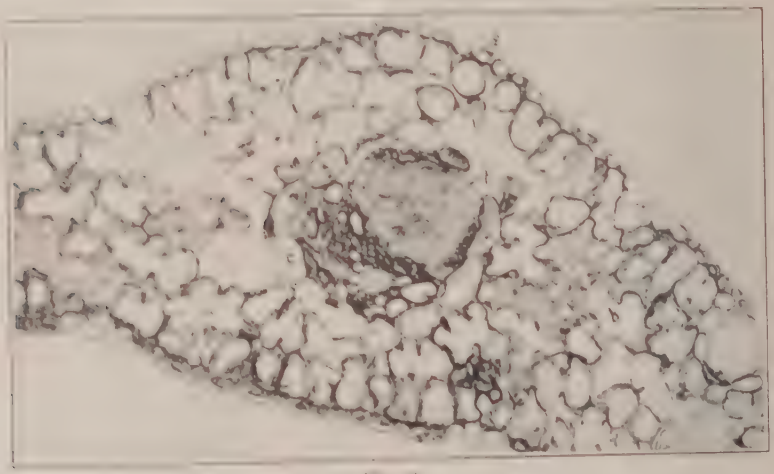


Fig. 2.

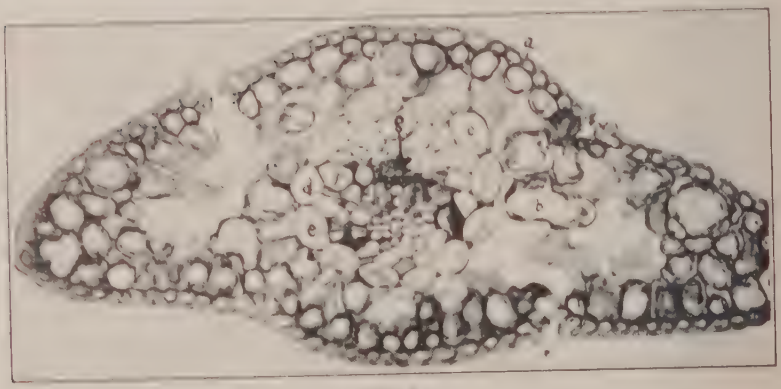
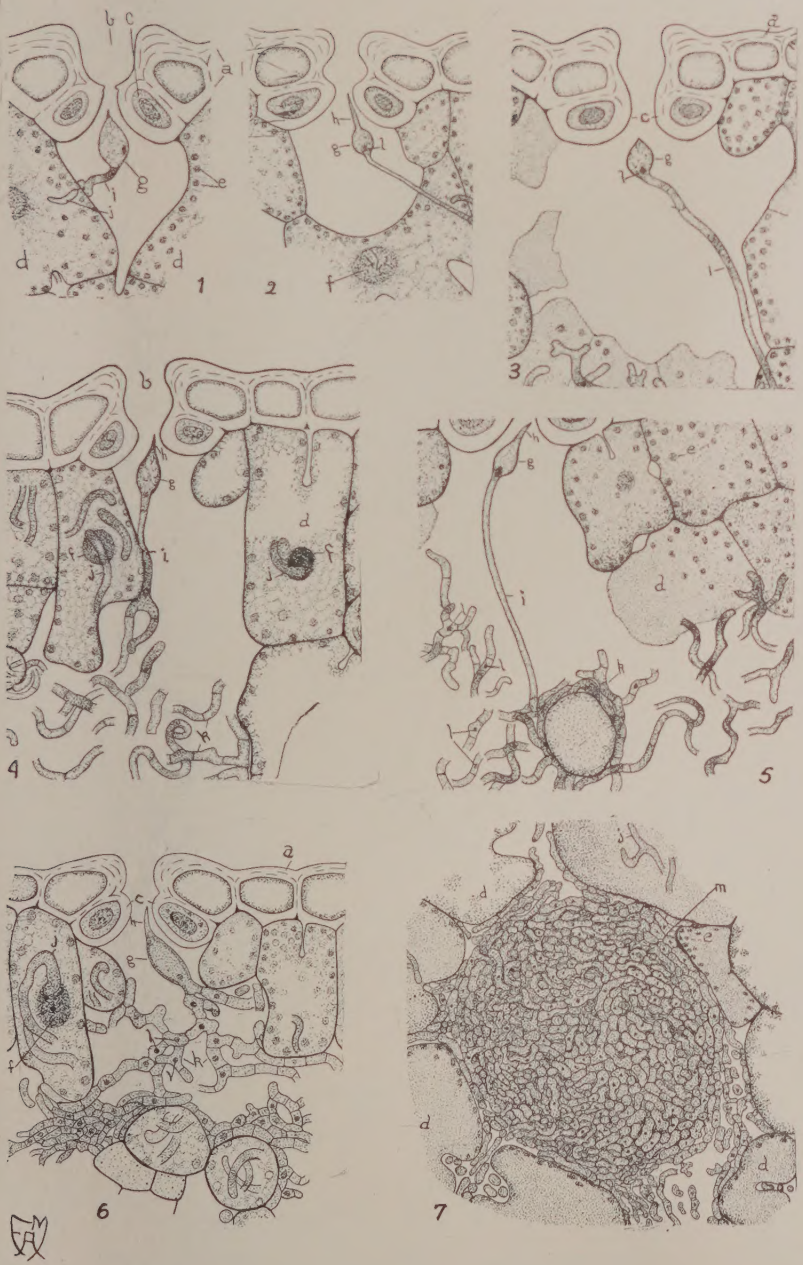


Fig. 3.

CROSS SECTIONS OF INFECTED PINE LEAVES.



CROSS SECTIONS OF INFECTED PINE LEAVES.

TELIAL STAGE AND ITS GERMINATION.